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Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Processable Molecularly Imprinted Star Polymer Based Sensors for Phosphorous Compounds					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
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[Page 1 of 2]

Respectfully submitted,

SIGNATURE

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Date 4/6/2004

REGISTRATION NO. 31,495

(if appropriate)

Docket Number: 2087-1331

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No. 2087-1331

In re application of: Glen E. Southard et al.

For: Processable Molecularly Imprinted Star Polymer Based Sensors for Phosphorous Compounds

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JHU/APL Docket No. 2087-1331

TITLE: Processable Molecularly Imprinted Star Polymer Based Sensors  
for Phosphorous Compounds

Invtors: G. E. Southard and G. M. Murray

#### 4. DESCRIPTION OF THE TECHNOLOGY:

- Written Description (Describe the invention in such detail that a person moderately skilled in the art may understand how to make and use it. In addition, attach any memos, etc. which show or describe the invention and identify them positively (i.e., by date and office symbol) below.)

Sensors for phosphorous compounds can be based on the luminescence of a lanthanide ion. This process is enhanced when the lanthanide ion has sensitizing ligands. If the ligands can be polymerized a molecularly imprinted polymer sensor can be made. However, the best sensitizing ligands, such as  $\beta$ -diketones may lose their ability to complex a lanthanide when a vinyl substituent is added. This means that some other means for polymerization must be found.

Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization is a free radical process that is controlled and ensures the formation of high polymer with a narrow molecular weight distribution. The process involves a chain transfer agent usually based on a dithioester. The process provides for a highly controlled system allowing the use of a broad spectrum of monomers and a highly controlled topology and morphology. Since this functionality does not involve conjugation to a  $\beta$ -diketone aromatic ring, it does not interfere with the ligand complexing ability.

We have prepared lanthanide containing imprinted RAFT polymers for the detection of several phosphorous compounds. The polymers has shown detection limits in the low parts per billion by luminescence spectroscopy and have been shown to be free of interference from phosphorous compounds that were not used as the imprinting species. We are now preparing star RAFT star polymers to provide fast kinetics, high solubility and processability.

The star polymer synthesis is given in the Figure on the following page.

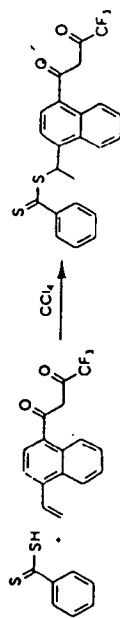


Figure 1. Preparation of the dithioester ligand.

The basic complex forms as follows, Figures 2 and 3.

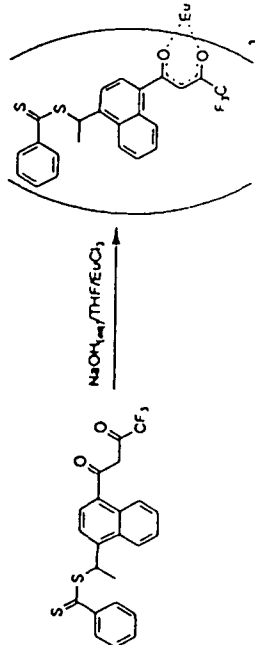


Figure 2. Preparation of the tris chelate.

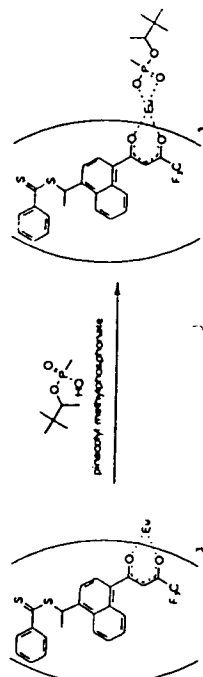


Figure 3. Conversion to the imprint complex. Polymerization is as follows in Figure 4.

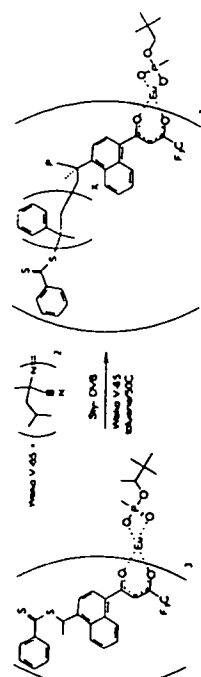


Figure 4. RAFT polymerization of the MIP for PMP

Polymers have been prepared by the RAFT polymerization method. Initial cleaning was attempted using three refluxing solvents, acetone, methanol and isopropanol. Isopropanol had the highest concentration of PMP after cleaning. Five milligrams of the isopropanol cleaned polymer was placed in a cuvette in xylene and examined by luminescence spectroscopy.

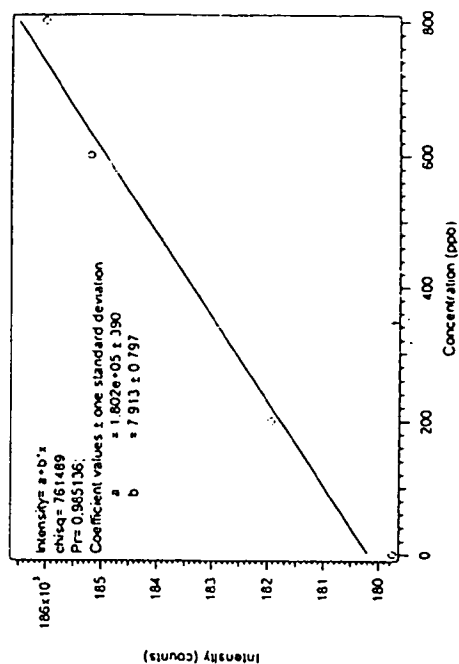
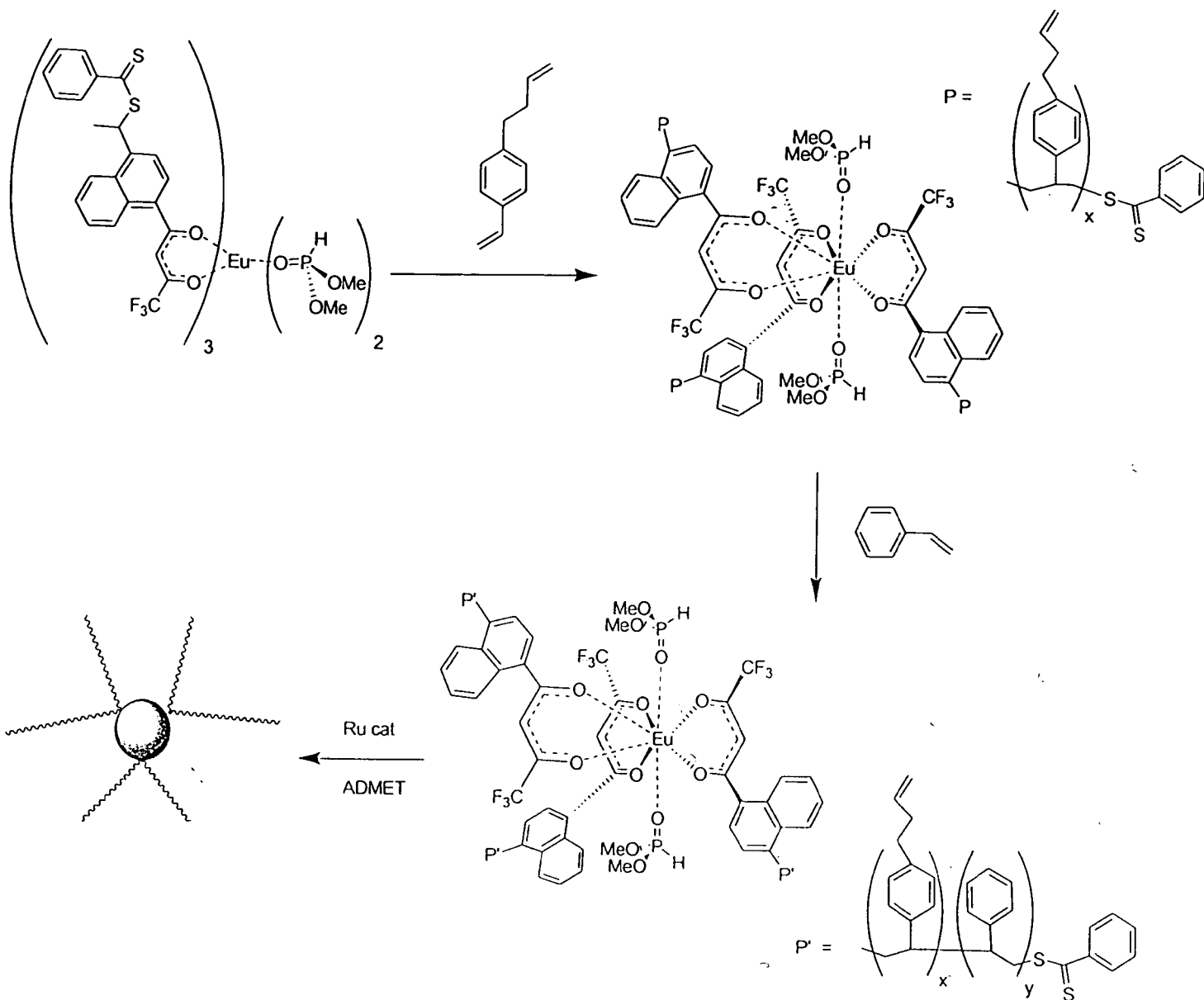


Figure 5. Calibration curve for the RAFT PMP MIP

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# **DEVELOPMENT OF IMPRINTED POLYMER SENSORS FOR THE DETECTION OF PINACOLYL METHYLPHOSPHONATE**

## **INTRODUCTION**

Molecular imprinting is a useful technique for making a chemically selective binding site. [1] The method involves building a synthetic polymeric scaffold of molecular compliments containing the target molecule with subsequent removal of the target to leave a cavity with a structural "memory" of the target. Molecularly imprinted polymers can be employed as selective adsorbents of specific molecules or molecular functional groups. Sensors for specific molecules can be made using optical transduction through chromophores residing in the imprinted site. The use of metal ions as chromophores can improve selectivity due to directional bonding. The combination of molecular imprinting and spectroscopic selectivity can result in sensors that are highly sensitive and nearly immune to interferences. [2]

## **BACKGROUND**

Spectroscopic sensing requires that a chromophore must be available and be influenced by the rebinding of the imprinted analyte. This can be accomplished in a variety of ways, the simplest of which is when the analyte is a chromophore itself. Sensors based on intrinsic analyte chromophores benefit from molecular imprinting by both selectivity and sensitivity enhancement. In terms of selectivity, molecules similar to the analyte are likely to have similar spectroscopic parameters that could be the source of interference in a conventional sensing strategy. The inability of an interferent to bind to the imprinted polymer allows discrimination. In terms of sensitivity, the analyte molecules can be effectively concentrated from the solution by the imprinted polymer. The degree of such concentration is controlled by the relevant binding constant. The target chromophore can be shielded from quenchers by the polymer matrix. The inclusion of non-complexing monomers isolates the chromophore, eliminates cross-talk and reduces concentration quenching. In the case of metal ion luminescence, the use of appropriate complexing ligands can enhance the intensity by several orders of magnitude through a variety of mechanisms.

Many metal ions are intrinsic chromophores and can be sensed by absorbance or luminescence. The transition metals exhibit colors based on inter-configurational d electron transitions. The intensity and the positions of the spectral bands can be influenced with a judicious choice of coordinating ligand. The molar absorptivities are less than those of organic chromophores due to the "forbidden" nature of the inter-configurational transitions, but proper choice of coordinating ligands can improve the absorptivity. Metal ions in the d block exhibit directed bonding and can provide both transduction and a proper geometry for analyte binding making useful extrinsic chromophores. By using an adducting strategy that changes the geometry of the reporter complex, significant changes in optical absorbance can be obtained.

Lanthanide ions are useful as intrinsic and extrinsic chromophores. Complexation by certain organic ligands enhances the luminescence intensity of the tripositive lanthanide, Ln(III) ions. The enhancement of luminescence has been explained by a ligand to metal energy transfer mechanism. This mechanism was derived from a series of investigations by Crosby, Kasha, and their co-workers. [3] Generally, when an excited triplet state of the coordinating ligand overlaps a lanthanide electronic level, the lanthanide luminescence can be effectively pumped by a larger cross section molecular absorbance, rather than by its own weak absorbance. This process is more efficient than direct absorption of light by the lanthanide due to the poor absorptivities of the lanthanides (formally atomically forbidden absorbance for the intra-configurational  $f \rightarrow f$  transition). A large number of organic ligands have been used to enhance lanthanide luminescence intensity. [4] When making an imprinted polymer sensor, the ligands must be chosen with sufficient affinity for the lanthanide, so as to coordinatively bind the ion in the polymer as well as provide intense luminescence. Many mixed ligand lanthanide complexes have been studied, providing clues to making a suitable sensor. Lanthanide ions have a thermodynamic affinity for a variety of anions, and this affinity can be exploited in making sensors for anions. Due to the relative hardness of lanthanides, the geometry of ligating atoms is a function of the steric strains imposed by the coordinating ligands. This is another avenue of exploitation for selectivity, since a careful selection of coordinating ligand can help define the line splitting by imposing specific site symmetry on the lanthanide in the resulting compound. By imposing certain coordination geometry on a complex, a large degree of change can be made to occur by ligand exchange, insuring a significant change in spectrum upon substitution.

## EXPERIMENTAL DETAILS

**Reagents.** Unless otherwise indicated, materials were obtained from commercial suppliers and used without further purification. Analytical reagent grade chemicals were used along with deionized water to prepare solutions. The chemicals used were obtained from Aldrich (Aldrich, Milwaukee, WI) or Fisher Scientific (Pittsburgh, PA).

**Instrumentation.** An in-house detection system was employed for screening lanthanide complexes that includes: an Ar Ion Laser, Model 543 Head and Model 170 Power Supply, (Omnichrome, Chino, CA) and an  $f/4$ , 0.5 meter monochromator (Chromex, Albuquerque, NM) equipped with a Model ST-6 CCD detector (Santa Barbara Instrument Group, Inc., Santa Barbara, CA). KestrelSpec software (Rhea Corp. Wilmington, DE) was used to operate the CCD and record the compound luminescence. A Cary 50 UV/VIS spectrophotometer (Varian, Walnut Creek, CA) was used to obtain absorbance spectra. The luminescence titrations were obtained using a Model QM-2 Fluorimeter/Phosphorimeter, (Photon Technologies International, Monmouth, NJ). Thermogravimetry was performed using a Model SDT 2960 Simultaneous DSC-TGA (TA Instruments, New Castle, DE). A Hewlett-Packard Model 5400 ICP-MS (Yokogawa Analytical Systems, Tokyo, Japan) was used to verify metal concentrations in all sample solutions. NMR was performed using a Model EFT 90 MHz spectrometer (Anasazi Instruments, Indianapolis, IN). The purity of synthesized organics was established using a Model QP 5050A GC/MS (Shimadzu, Columbia, MD). The Heck coupling reaction

was performed with either a Model HC 677 100 mL reactor (Parr Instruments, Moline, IL) or an LC series 300 mL reactor (Pressure Products Industries, Warminster, PA).

**Ligand Synthesis.** The procedures followed for ligand synthesis developed in the previous year were amended and modified to improve yield and allow for the production of new monomers to overcome problems with vinyl-substituted  $\beta$ -diketones. One specific improvement was a new form of Heck coupling that reduced losses to over reduction (ethyl substitution) and inadvertent polymerization.[5-11] The other issue was that the earlier form of Heck coupling did not function with the electron rich trifluoromethyl substituted  $\beta$ -diketones. The trifluoromethyl and halogen substituted  $\beta$ -diketones, 4-bromo naphthoyltrifluoroacetone and 5-bromothienoyltrifluoroacetone, were synthesized by condensation of acetyl bromoarene with ethyltrifluoroacetate under basic conditions in ether. It was found that potassium tert-butoxide gave better results for 4-bromo naphthoyltrifluoroacetone, while sodium methoxide was an excellent base for generating 5-bromothienoyltrifluoroacetone.

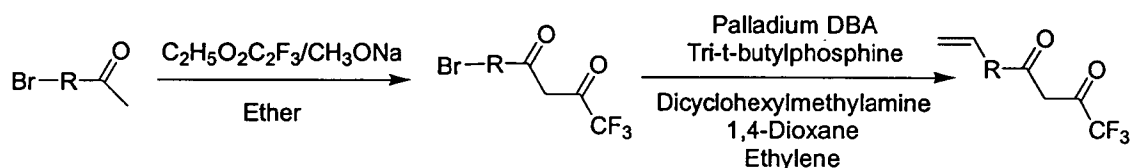


Figure 1. General synthesis of vinyl substituted aryl-4,4,4-trifluoro-butane-1,3-diones.

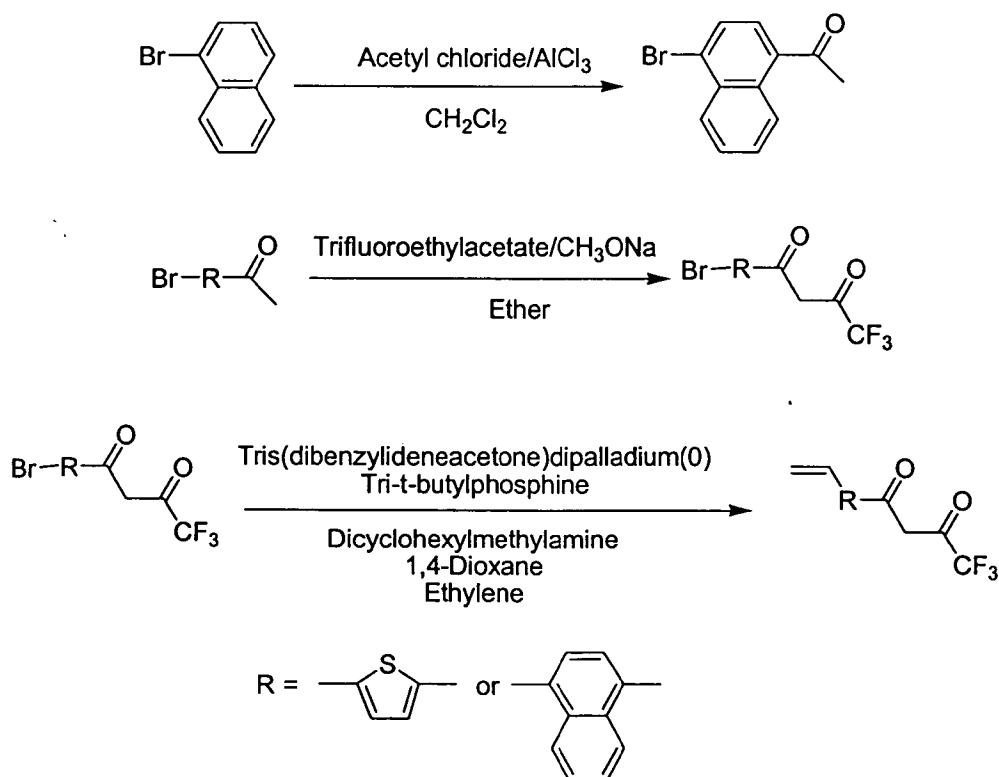


Figure 2. Steps involved in the synthesis of vinyl-naphthoyltrifluoroacetone and vinyl-thienoyltrifluoroacetone.

There were difficulties in getting good complexation and adduct formation with vinyl substituted  $\beta$ -diketone ligands. A path to an imprinting complex that does not involve ordinary free radical polymerization was sought. Reversible Addition Fragmentation Transfer (RAFT) polymerization is a free radical process that is controlled and ensures the formation of high polymer. The process involves a chain transfer moiety usually based on a dithioester. Since this functionality does not involve conjugation to the  $\beta$ -diketone aromatic ring it does not interfere with the ligands complexing ability, Figure 2.

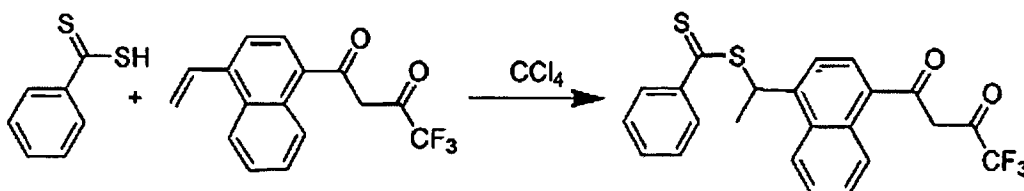


Figure 3. Preparation of the dithioester ligand.

Ligands prepared with the dithioester substituents seen in Figure 3 formed stable complexes with europium (III) and formed stable adduct complexes with PMP.

**Complex Synthesis.** In order to prepare polymers it is necessary to first prepare a complex of europium (III), sensitizing ligands and analyte. In the preparation of model complexes this was straightforward, Figure 4. It turns out that the substitution of a vinyl group on the aromatic rings of the  $\beta$ -diketone ligands reduced their ability to form complexes and require different methodologies. The first and simplest methodology was

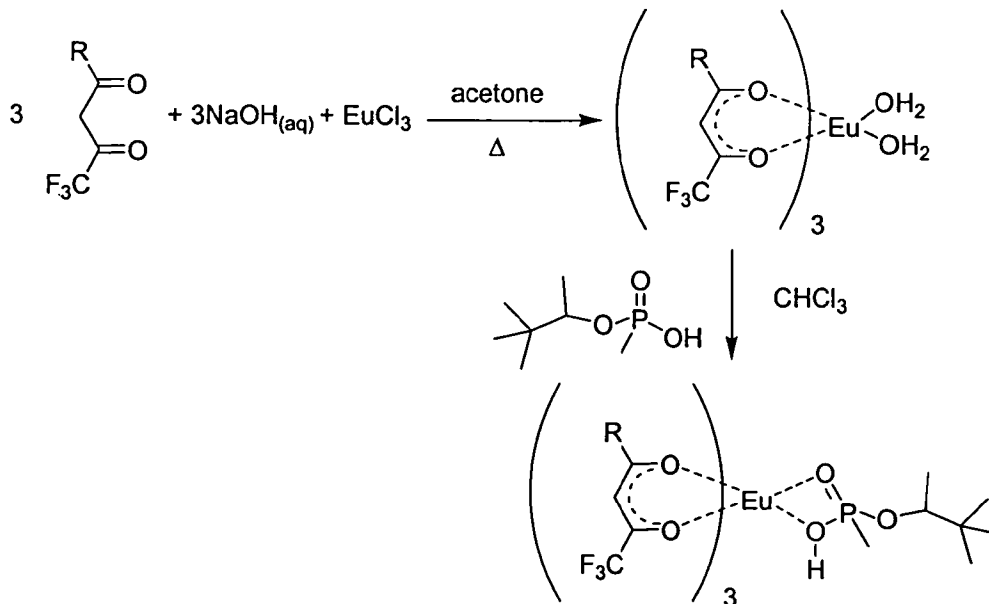


Figure 4. Synthesis of the tris  $\beta$ -diketone europium complex, followed by a description of PMP adduct formation in chloroform.

to prepare a mixed ligand complex with two fluorinated ligands and the one vinyl-substituted  $\beta$ -diketone that still complexes europium (III), 3-vinyldibenzoylmethane. In this manner the fluorinated ligands made europium (III) hard enough to form

phosphonate adducts and the 3-vinyldibenzoylmethane made the complex polymerizable. The presence of water of hydration was verified using thermo-gravimetric analysis. The thenoyltrifluoroacetone complex had two water molecules of hydration as expected, to give europium (III) the normal coordination number of nine. The vinyl-naphthoyltrifluoroacetone complex showed an additional two molecules of water per complex that are suspected to be lattice water.

As stated above the dithioester substituted ligand behaved in the same manner as the unsubstituted trifluoromethyl ligands. The formation of the europium complex and the suspected route of adduct formation is shown in Figures 4 and 5. The basic complex forms as follows, Figures 5 and 6.

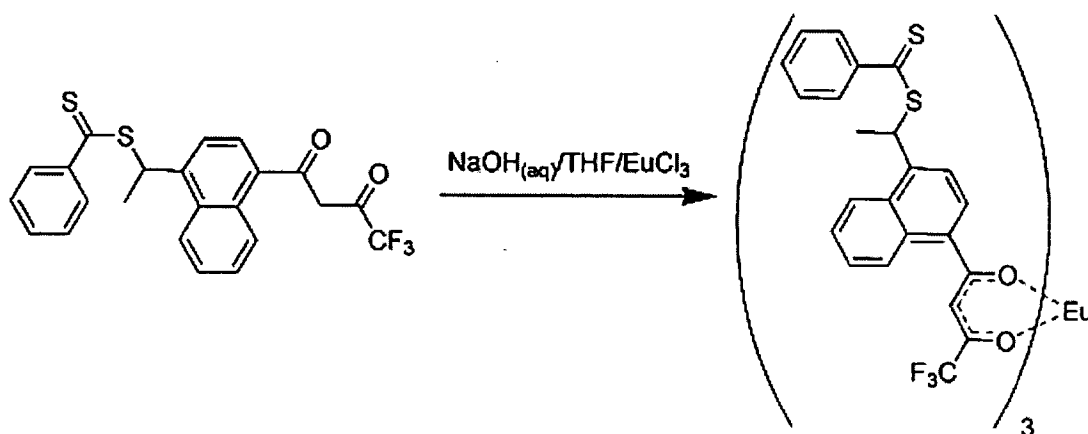


Figure 5. Preparation of the tris chelate.

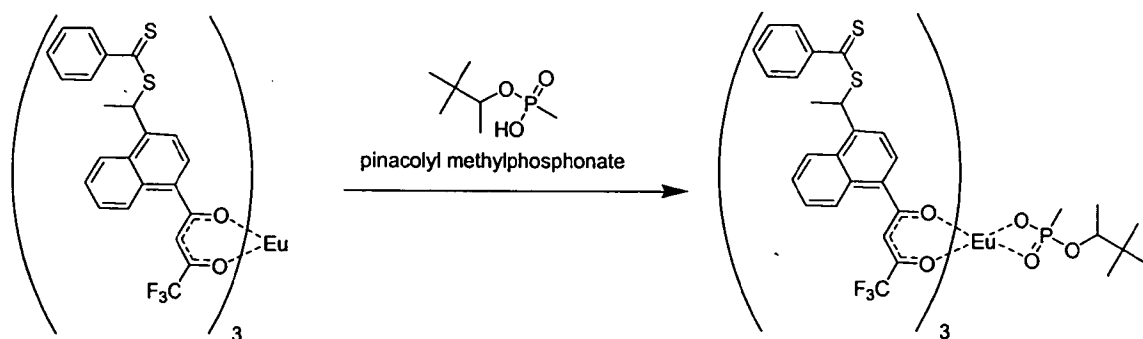


Figure 6. Conversion to the imprint complex.

**Polymer Synthesis.** MIPs were prepared for pinacolyl methylphosphonate, (PMP) using a mixed ligand approach (3-vinyldibenzoylmethane and naphthoyltrifluoroacetone) in a matrix of methyl methacrylate (MMA) and ethylene glycol dimethacrylate (EGDM) using toluene as a porogen. The amount of the crosslinker, EGDM, was 80 weight %. The polymers produced were ground in a cryo-grinder to a fine powder. Several cleaning methods were characterized so as to best remove the imprint molecule without compromising the integrity of the MIP. The washes were evaluated using GC/MS. Of

the systems tested, it was determined that unadulterated methanol was the best solvent for removing PMP without compromising polymer integrity.

The mixed ligand approach was followed by the RAFT approach. The polymers were made using both styrene and methyl methacrylate as matrix monomers. The porosity of the resultant polymers was limited. The procedures were repeated using methoxyethanol in place of toluene as the solvent. The polymers prepared with methoxyethanol ground easier and were more miscible with xylene.

**General Procedure for RAFT polymerizations of  $L_3EuPMP$  with methacrylate:** EGDMA (16 mmol), MMA (8 mmol), toluene (4 mL), Wako V-65 (0.044 mmol), PMP (0.029), and  $L_3Eu$  (0.029 mmol) were placed into a disposable glass reaction flask equipped with a stir bar. The solution was subjected to three freeze/pump/thaw cycles with argon backfill. The solution was placed into an oil bath heated to 60°C for 18 hours before the solvent and unreacted monomer were removed by heating to 60°C while under vacuum (0.5 torr) for 4 hours. The salmon-colored polymer was ground with a freezer mill to a fine powder.

**General Procedure for RAFT polymerizations of  $L_3EuPMP$  with styrene:** DVB (22 mmol), Sty (11 mmol), toluene (4 mL), Wako V-65 (0.044 mmol), PMP (0.029), and  $L_3Eu$  (0.029 mmol) were placed into a disposable glass reaction flask equipped with a stir bar. The solution was subjected to three freeze/pump/thaw cycles with argon backfill. The solution was placed into an oil bath heated to 60°C for 18 hours before the solvent and unreacted monomer were removed by heating to 60°C while under vacuum (0.5 torr) for 4 hours. The salmon-colored polymer was ground with a freezer mill to a fine powder.

The polymerization procedure for the styrenic RAFT polymer is illustrated in Figure 7.

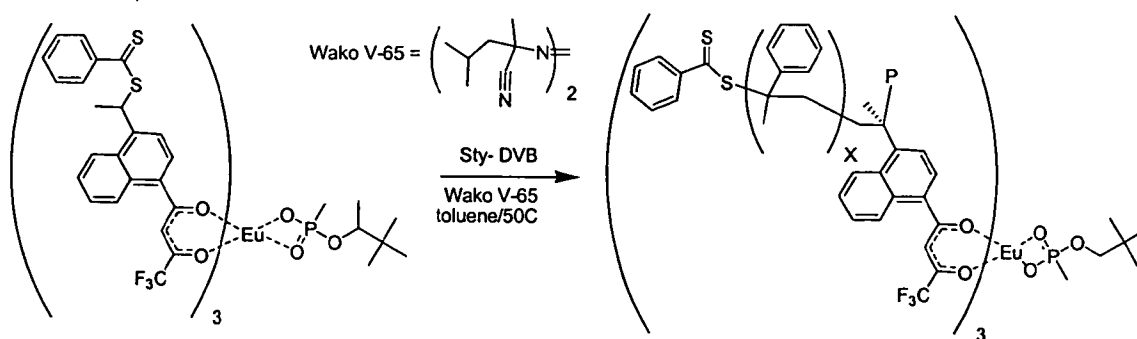


Figure 7. RAFT polymerization of the MIP for PMP.

## RESULTS AND DISCUSSION

Metal ion complexes can form a binding site that exhibits both a spectral signature and can be expected to survive incorporation into a polymer. A solution analogue of the binding site can be prepared and the utility for use in imprinting may be established. For a d block complex, adduction may be characterized by obtaining absorbance, or in some

cases, luminescence spectra. The spectral changes that accompany adduct formation are either changes in band position or intensity. Lanthanide based transduction complexes are usually based on luminescence intensity. If, as in the case of a lanthanide-based reporter, luminescence varies with the degree of analyte inclusion, a luminescence titration will reveal stoichiometry.

We made improvements to the binding site we used previously for organophosphates. The earlier work utilized a relatively poor sensitizing ligand, vinyl benzoate.[2] We have spent a considerable amount of time and effort synthesizing a variety of vinyl substituted  $\beta$ -diketones. In the course of this work we have discovered that some of the vinyl substituted  $\beta$ -diketones exhibit significantly different complexing abilities relative to their unsubstituted parents. The position of substitution also had a significant effect. We have found two complexing ligands, thenoyltrifluoroacetone and naphthoyltrifluoroacetone, that are suitable for the production of organophosphate adducts. Since the tris chelates are coordinatively unsaturated they are typically hydrates and thus the luminescence intensity of the organophosphate adducts increases. However, to produce polymers using these ligands a mixed ligand approach using 3-vinyldibenzoylmethane was required.

Although, a thioester-substituted  $\beta$ -diketone ligand was eventually chosen for the sensor, many vinyl-substituted  $\beta$ -diketones were found to have adverse properties. For example, methylene substituted  $\beta$ -diketones exist as three tautomers, as observed by NMR spectra. The most stable tautomer gives isolated  $\pi$  system, resulting in unusual chemistry. The ligands no longer complex well and do not sensitize luminescence. The resulting compounds were insensitive towards the analyte. The initial choice of ligand monomer was a vinyl-substituted form of dibenzoylmethane. Problems associated with this choice began when it was discovered it was all but unobtainable by trying to couple vinyl-acetophenone to vinyl-methylbenzoate using sodium hydride. After the appropriate synthetic route was established, the potentially most attractive variant for imprinting, 4,4'-divinyldibenzoylmethane proved too sterically hindered to form viable complexes. It had been hoped that two connections to the polymer chain would provide the most stable binding site. The excitation spectrum of the europium (III) complex showed a maximum at 430 nm, a wavelength easily obtainable using a light emitting diode (LED). When other forms of dibenzoylmethane were employed, the complexes partially dehydrated in organic solvents. While this was reversible, it was unexpected and caused confusion when attempting to interpret spectra.

Harry Brittain [12] developed a luminescence titration method for the determination of the stoichiometry of europium (III)  $\beta$ -diketone complexes and phosphate esters. In his work, adducts were formed in a 1:1 mole ratio for tris europium (III)  $\beta$ -diketone complexes, having at least one trifluoromethyl substituent. We applied this method to determine the stoichiometry of the adduct of pinacolyl methylphosphonate (PMP) and europium (III) tris naphthoyltrifluoroacetone ( $\text{Eu}(\text{NTFA})_3$ ) in chloroform. The excitation wavelength was 360 nm and the slits of the excitation and emission monochromators were set at 2 nm. A triangular cell, instead of the 180° geometry used by Brittain, was used to reproduce the experiment in a commercial fluorimeter. So, instead of 3.0 mL

aliquots, 1.5 mL aliquots were employed. The spectra produced by the addition of partial equivalents of PMP to  $\text{Eu}(\text{NTFA})_3$  up to 1.05 equivalents is presented in Figure 8. Unlike the phosphate esters studied by Brittain, addition of PMP beyond one equivalent causes a loss of  $\beta$ -diketone, as seen in Figure 9. Thus, instead of reaching a sustained maximum intensity of luminescence, the luminescence increases up to one equivalent and then decreases. The plot does show that the 1:1 complex has the greatest luminescence intensity. The complex should be suitable for an imprinted sensor, since, once formed the amounts of PMP that the sensor will encounter will never approach the stoichiometric value.

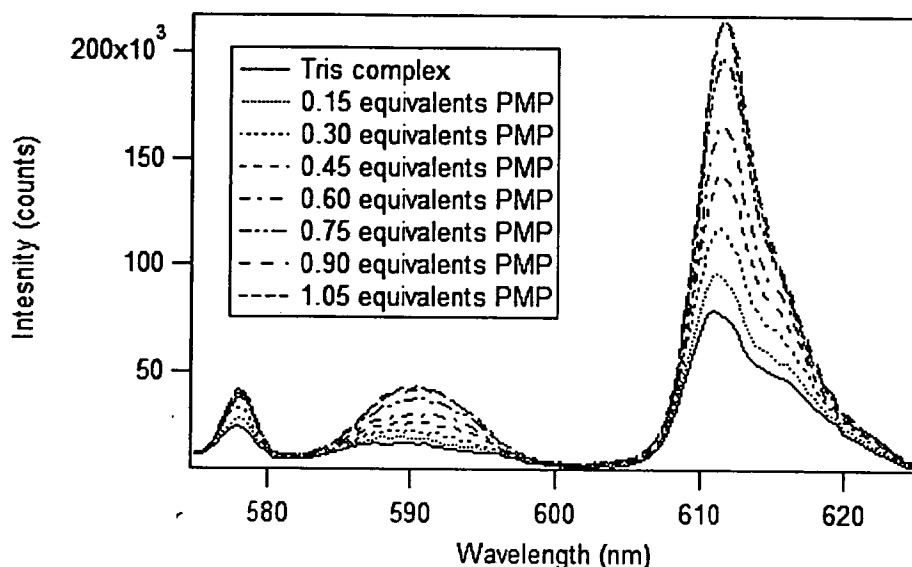


Figure 8. Luminescence spectra of  $\text{Eu}(\text{NTFA})_3$  with addition of PMP in chloroform.

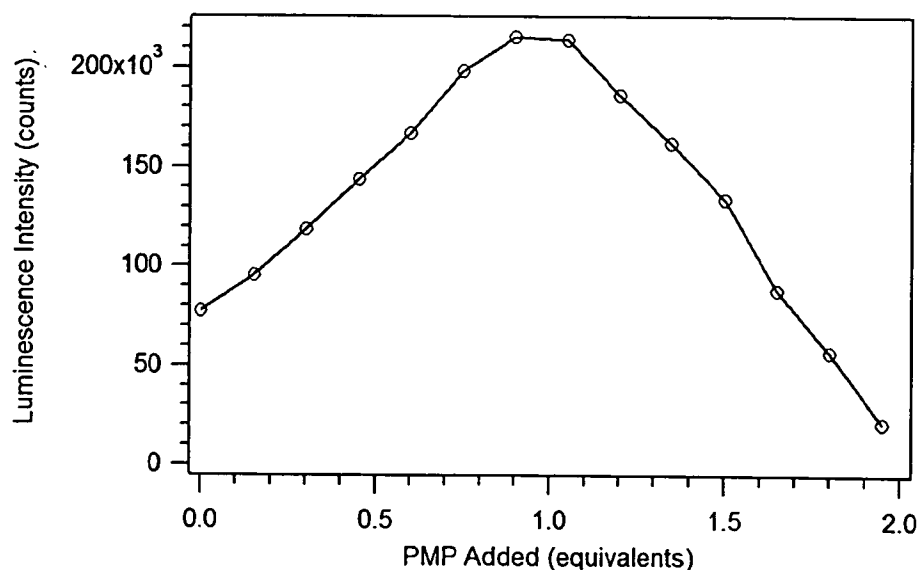


Figure 9. Plot of the intensity of the 611 nm luminescence peak versus equivalents of PMP added.



The inclusion of a luminescent chromophore into an organic polymer may be complicated by background luminescence. When  $\text{Eu}(\text{NTFA})_3$  was incorporated into a styrene copolymer, the continuous wave (CW) luminescence spectrum showed a large background, Figure 8. This background is observed when using a broad band light source for excitation and not observed when the luminescence is excited by an Ar ion laser at 465.8 nm. Since our intent is to make small portable sensors, the ability to pump a broad allowed ligand band with a small light source such as a LED, as opposed to pumping a sharp weak europium (III) absorbance with a large laser, is desired. The background was eliminated by using a pulsed light source and gated detection. The excitation spectra are not very different except there are some spikes due to the pulsed lamp's output. The emission spectrum now has a flat background, showing the absence of scattered light and matrix fluorescence. The time-resolved spectra were obtained by delaying signal collection until the excitation light had been off for 30 microseconds and integrating for a period of 1 millisecond. The slits of the excitation and emission monochromators were set at 2 nm, and the polymers were mounted on glass plates, cut to fit the cuvette holder of the phosphorimeter. The long luminescence lifetimes exhibited by the lanthanide are compatible with less expensive and relatively slow electronics.

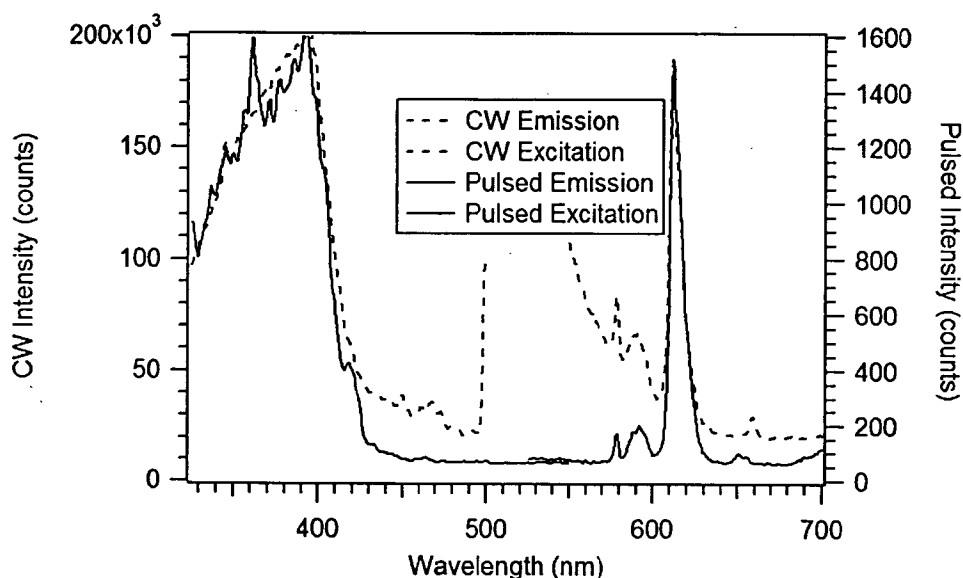


Figure 9. The use of time resolved luminescence to discriminate against background luminescence and scattered light.

### Mixed Ligand Approach

MIPs were prepared for using a mixed ligand approach (3-vinyldibenzoylmethane and naphthoyltrifluoroacetone) in a matrix of methyl methacrylate (MMA) and ethylene glycol dimethacrylate (EGDM) using toluene as a porogen. This approach was required since the vinyl substituted ligands made weaker complexes than their unsubstituted counterparts. Cleaning the mixed ligand polymer resulted in loss of significant amounts of europium. This is a result of the statistical nature of the ligand substitution such that some of the imprint complex is not bound to the polymer. When the polymers were applied to rebinding studies the result was a loss in luminescence as the PMP was added.

This was interpreted as dissociating the complex, due to the weaker interaction with the vinyl substituted ligand. This approach was viewed as unsatisfactory so a different approach was followed.

## RAFT Polymers

Polymers were prepared by the RAFT polymerization method. Initial cleaning was attempted using three refluxing solvents, acetone, methanol and isopropanol. Isopropanol had the highest concentration of PMP after cleaning and was used in all subsequent cases. In a first study, five milligrams of the isopropanol cleaned polymer was placed in a cuvette in xylene and examined by luminescence spectroscopy. Ten minute intervals were used between additions. A calibration curve generated by this process is presented as Figure 10. The curvature of the calibration curve suggests that either there were insufficient sites in the polymer to cover the dynamic range or that ten minute intervals are not long enough to reach equilibrium.

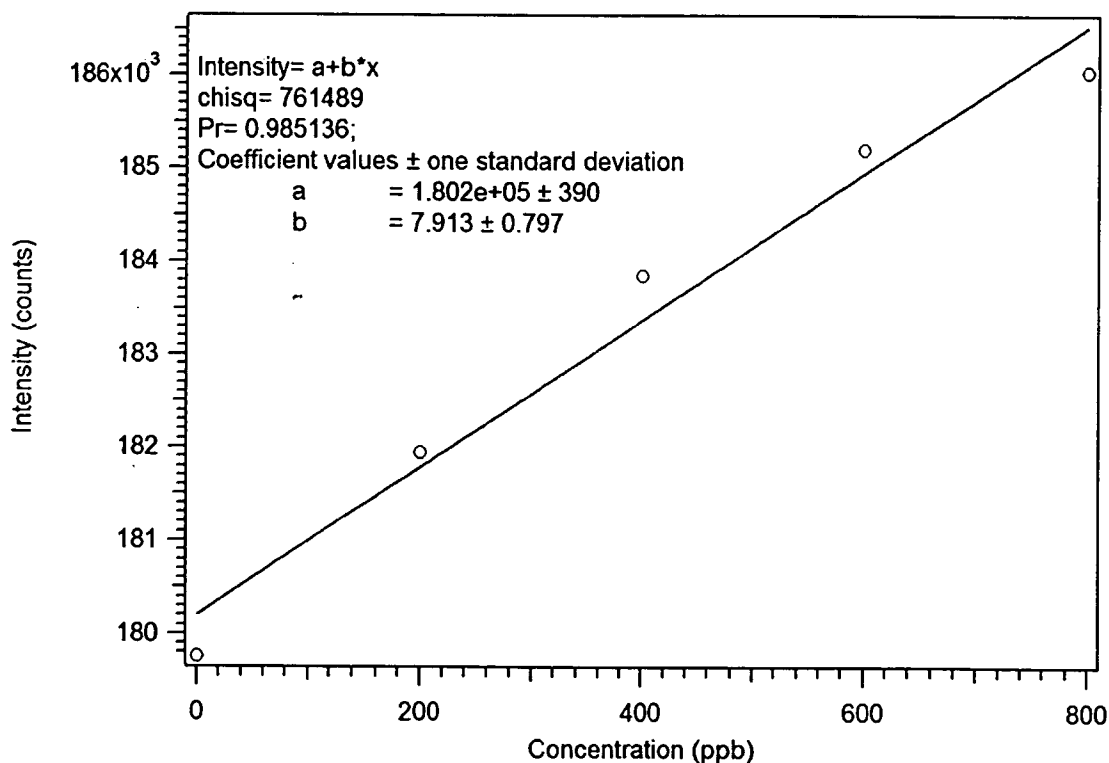


Figure 10. Calibration curve for the RAFT PMP MIP.

In order to assure complete removal of PMP, the polymer was next cleaned by Soxhlet extraction with isopropanol. The concentration of PMP in the solvent was measured hourly until a steady state concentration was achieved. Extraction of the RAFT polymers does not liberate any europium, showing that the complexes are fully incorporated into the polymer. Since the incorporation of complex is better, smaller amounts of polymer can be used to get good sensitivity and dynamic range. A second calibration curve was generated using the RAFT polymer and longer equilibration times in order to ascertain whether equilibration is slow (site accessibility is kinetically hindered) or if the cleaning

was inadequate and too few sites were available. Since the fluorimeter has been used extensively and there is evidence of lamp intensity decline a new lamp was ordered. Also, a control solution of the model compound was used to adjust for instrument changes.

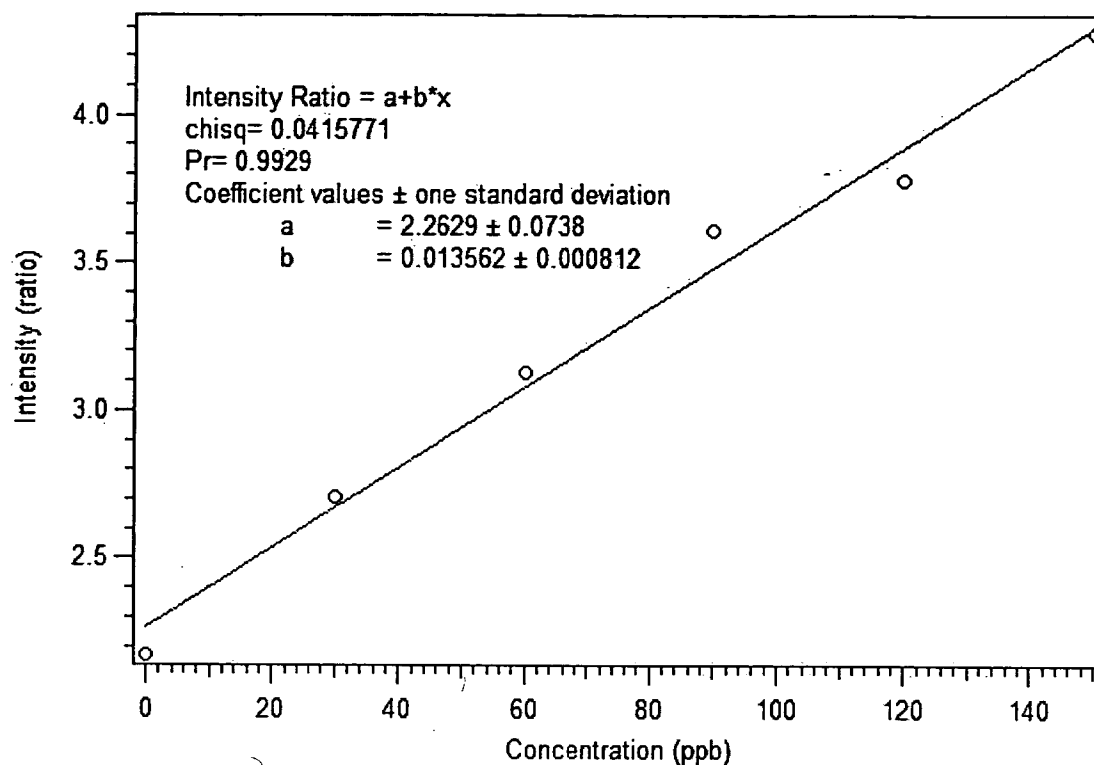


Figure 11. Calibration curve using longer time intervals and a ratio to standard showing much improved sensitivity.

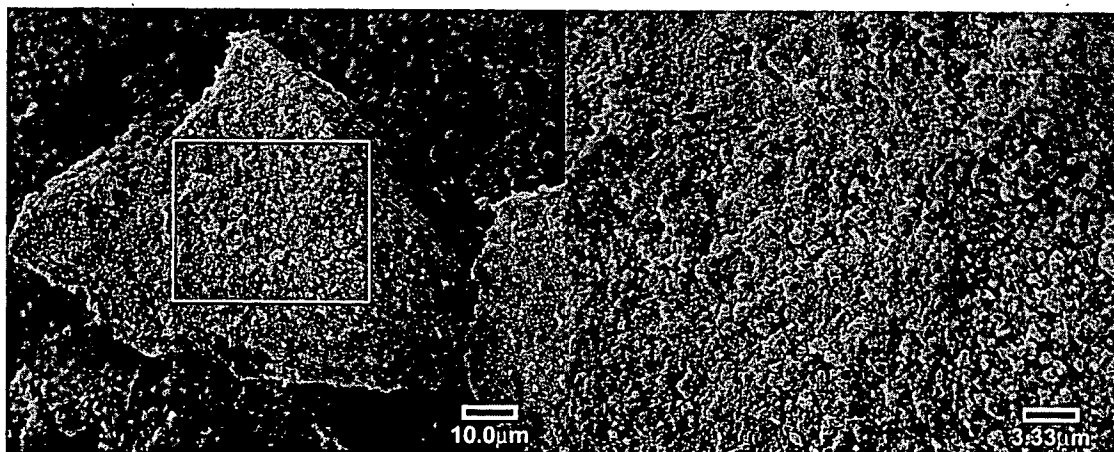


Figure 11. Scanning electron micrographs of a large particle of the mixed ligand polymer (left 1000 X, right 3000 X).

The slow kinetics associated with the first RAFT polymers was suspected to be caused by a lack of porosity or surface roughness. This hypothesis was tested by the use of a

scanning electron microscope to investigate the particle's surfaces. As seen in Figure 12, the mixed ligand polymer particles have a rough surface conducive to fairly rapid equilibration. The following Figure, 13, shows that the surface of the RAFT polymer is much smoother. This effect is attributed to the much more even and controlled reaction of RAFT polymerization. In order to improve the surface roughness and porosity we changed the porogen/solvent to methoxyethanol, a highly polar solvent to induce phase separation during polymerization, Figure 14. The particles of the new solvent polymer were smaller. The RAFT polymer prepared using methoxyethanol was observed to form an opaque light pick polymer that readily cracked when solvent was removed. The polymer ground to a very fine powder and appeared to completely dissolve in xylene, showing that the refractive indices were matched.

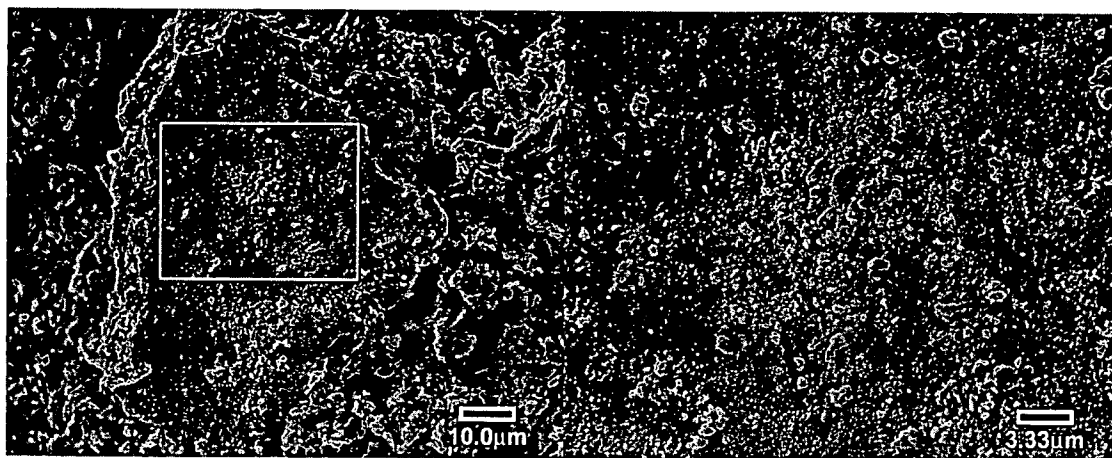


Figure 12. Scanning electron micrograph of a large particle of the RAFT polymer using toluene as the solvent (left 1000 X, right 3000 X).

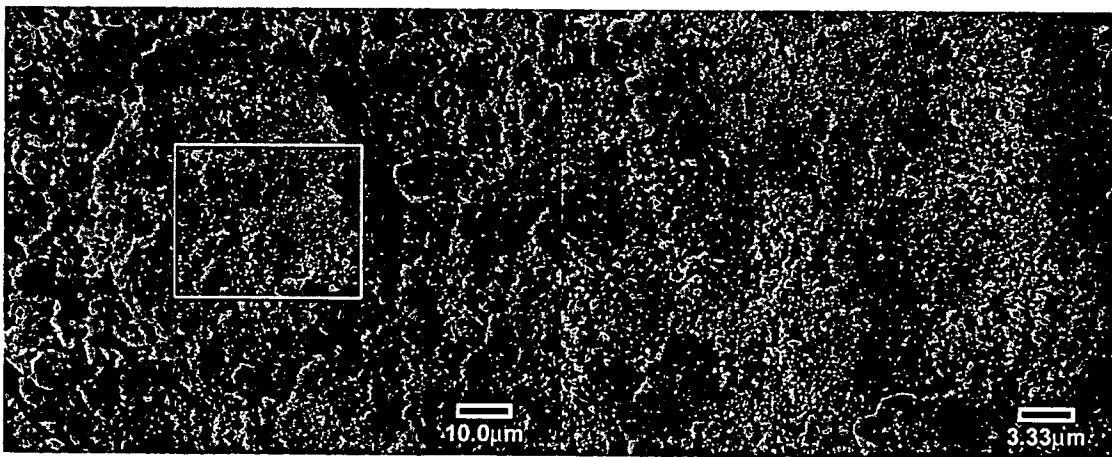


Figure 13. Scanning electron micrograph of a large particle of the RAFT polymer using methoxyethanol as the solvent (left 1000 X, right 3000 X).

## CONCLUSIONS

Molecularly imprinted polymers with good sensitivity (LOD's in the low ppb range) and very high selectivity (no interference from near identical interferents) have been prepared

using RAFT polymerization. The polymers were designed to be used in a liquid light guide based sensor system. The polymers have the same refractive index as xylene and both the polymer and xylene should work well in a Teflon AF light guide. Polymers prepared using methoxyethanol have better porosity and exchange kinetics than do polymers prepared using toluene as solvent and porogen.

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## BACKGROUND

Molecular imprinting is a process for making selective binding sites in synthetic polymers. The process may be approached by designing the recognition site or by simply choosing monomers that may have favorable interactions with the imprinting molecule. To successfully apply the methodology to production of a chemical reporter requires the designed approach. The process involves building a complex of an imprint molecule and complimentary polymerizable ligands. For a self-contained reporter, at least one of the molecular compliments must exhibit a discernable physical change associated with binding. This change in property can be any measurable quantity, but a change in luminescence is the most sensitive and selective analytical technique. By copolymerizing the complexes with a matrix monomer and a suitable level of cross-linking monomer, the imprint complex becomes bound in a polymeric network. The network must be mechanically and chemically processed to liberate the imprinting species and create the binding site. The design of the binding site requires chemical insight. These insights are

derived from studies of molecular recognition and self-assembly and include considerations of molecular geometry, size and shape, as well as molecule-to-ligand thermodynamic affinity.

The standard approach to molecular imprinting does not lend itself to the production of processable materials. Reversible Addition-Fragmentation Transfer (RAFT) polymerization is a free radical process that is controlled and ensures the formation of high polymer with a narrow molecular weight distribution. The process involves a chain transfer agent usually based on a dithioester. The process provides for a highly controlled system allowing the use of a broad spectrum of monomers and a highly controlled topology and morphology. By using this methodology we can prepare soluble molecularly imprinted macromolecules. The physical form of the soluble macromolecule is called a "star polymer" due to several straight chain polymers emanating from a central cross-linked core. The cross-linked core holds the imprinted site.

## APPROACH

The basis for this approach lies in the ability to imprint a central core of a macromolecular complex with chemical recognition. This differs from normal molecular imprinting by avoiding an insoluble cross-linked bulk polymer. The process takes advantage of a new technique in controlled free-radical polymerization, the RAFT polymerization method. The RAFT method of polymerization allows the production of polymers in stages, by a graft of one set of monomers after another. In this manner, a core complex of RAFT polymerizable ligating monomers and imprint molecule is first reacted with a cross-linkable monomer. The cross-linkable monomers have one vinyl and one butenyl substituent. The vinyl substituent is incorporated into the polymer and the less reactive butenyl substituent is left unreacted. Excess cross-linkable monomer is removed and a matrix monomer is added. Chains of matrix monomer are incorporated as the arms of the star. Excess matrix monomer is removed and a catalyst is added to tie the butenyl substituents together to form a cross-linked core. The imprint molecule is removed using an appropriate solvent, resulting in a soluble imprinted macromolecule. Due to the living nature of the RAFT reagent, it persists at the end of the chain and the macromolecules can be processed and subsequently chemically bound in whatever matrix is provided. This approach to soluble imprinted macromolecules has only recently been developed at JHU/APL. The ability to form soluble imprinted macromolecules opens the door to making readily processable imprinted materials.

## PRIOR WORK

We have been preparing molecularly imprinted polymers as sensors for the determination of organophosphates for several years. In our first work we used a benzoate complex to bind pinacolyl methylphosphonate (PMP), the hydrolysis product of the nerve agent Soman. The sensor was designed as a water monitor and since the agent readily hydrolyzes in water and the hydrolysis product is innocuous imprinting was based on this compound. The sensor was able to detect PMP in water at concentrations in the low parts per billion. Following that project we have developed better polymerizable lanthanide

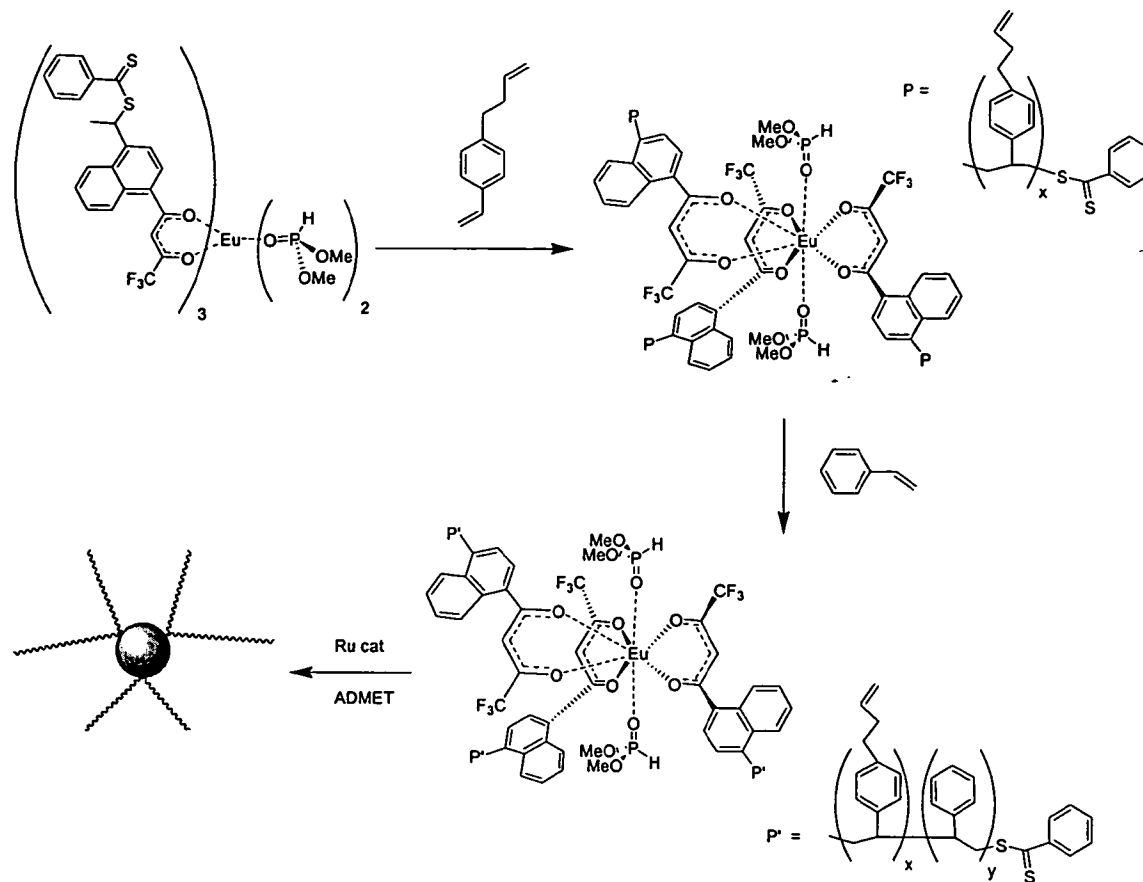


Figure 1. Star polymers formed by the RAFT method for the selective detection of an organophosphorous compound.

coordinators to improve the sensitivity and selectivity of the sensor. The new coordinators have been found to make the  $\text{Eu}^{3+}$  center sufficiently electropositive so as to bind organophosphonates that do not have an ionizable proton to form an ionic bond to the lanthanide. The new coordinators were also designed to improve luminescence yields and lower the limits of detection. This work would have application to the production of an instrument for detecting these agents.

We have since prepared lanthanide containing imprinted RAFT polymers for the detection of several phosphorous compounds. The polymers has shown detection limits in the low parts per billion by luminescence spectroscopy and have been show to be free of interference from phosphorous compounds that were not used as the imprinting species. We are now preparing star RAFT star polymers to provide fast kinetics, high solubility and processability. The technology is ready to be adapted to other promising applications as taggants, sequestering agents and catalysts.





# Objectives

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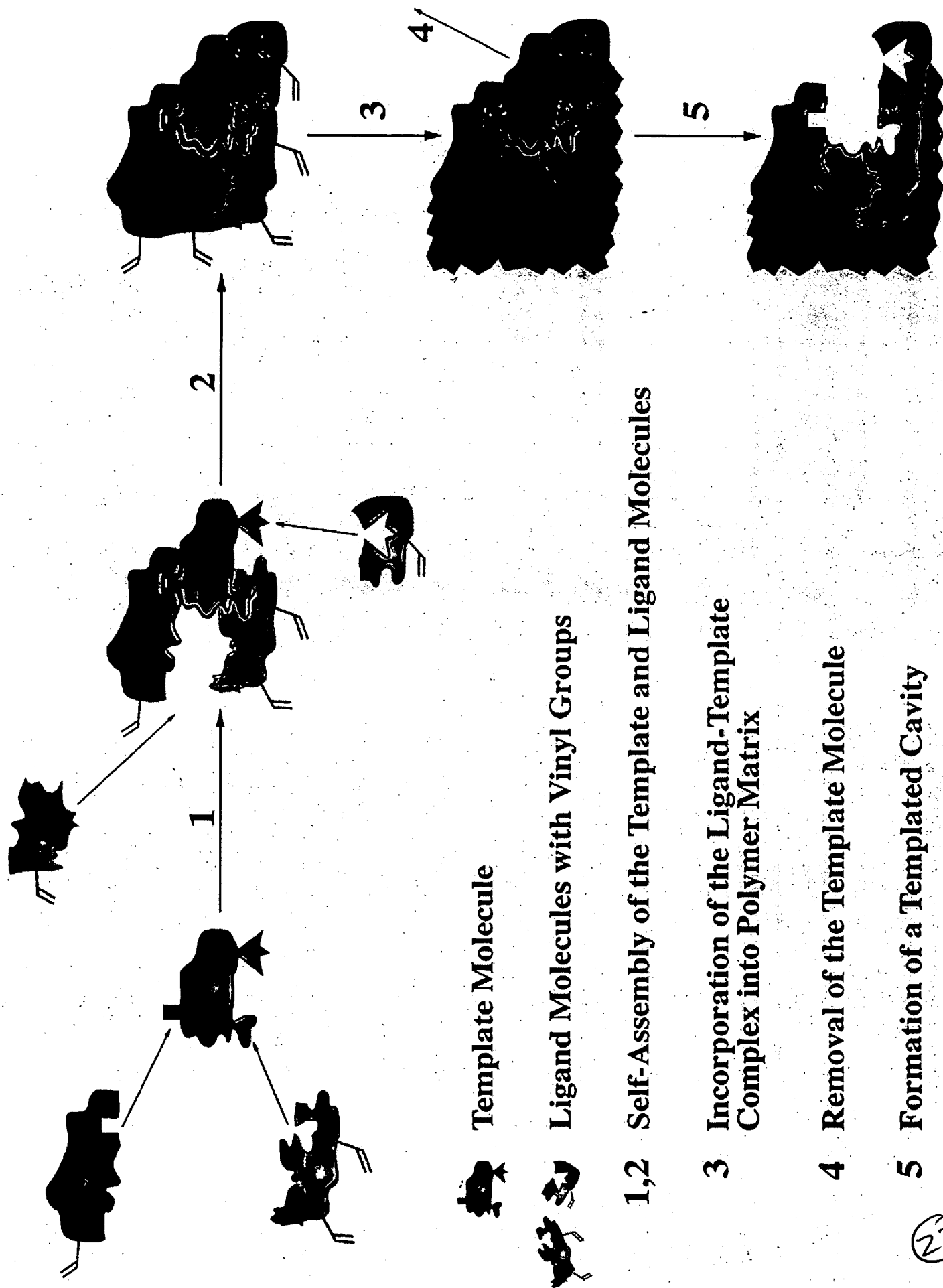
- Sense pinacolylmethylphosphonate (PMP) by fluorescence enhancement
  - » Use lanthanide metal as fluorophore
- PMP specific
  - » Use molecularly imprinted polymers (MIPs) for specificity
- High sensitivity
  - » Conjugated  $\beta$ -diketones
- Versatile

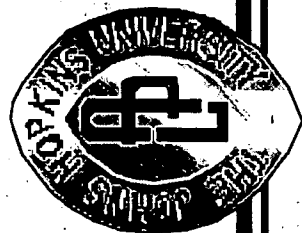


# Use of $\text{Ln}^{3+}$ Chromophore

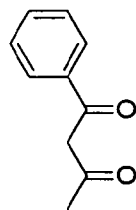
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- 1) Lanthanide ions exhibit narrow lines and long luminescence lifetimes (selectivity).
- 2) Lanthanide ions have an affinity for certain functional groups.
- 3) Lanthanide spectra can be used to determine the association of a complexing ligand.
- 4) Sensitizing ligands can enhance the luminescence of the lanthanide and provide improved light absorption and radiative efficiencies.

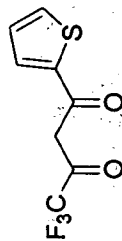




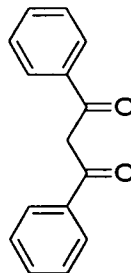
# Major Players



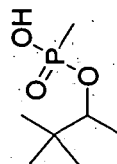
Benzoylacetone (BzA)



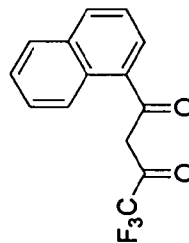
Thienyltrifluoroacetone (TTFA)



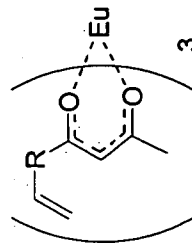
Dibenzoylmethane (DBM)



Pinacolylmethylphosphonate (PMP)



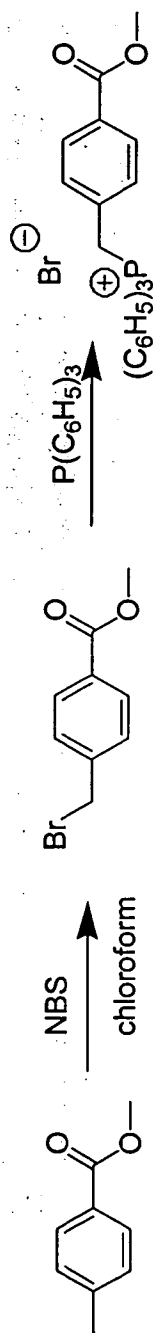
Naphthyltrifluoroacetone (NTFA)



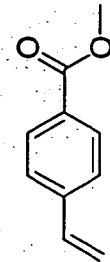
Complex



# Improved Ligand Synthesis



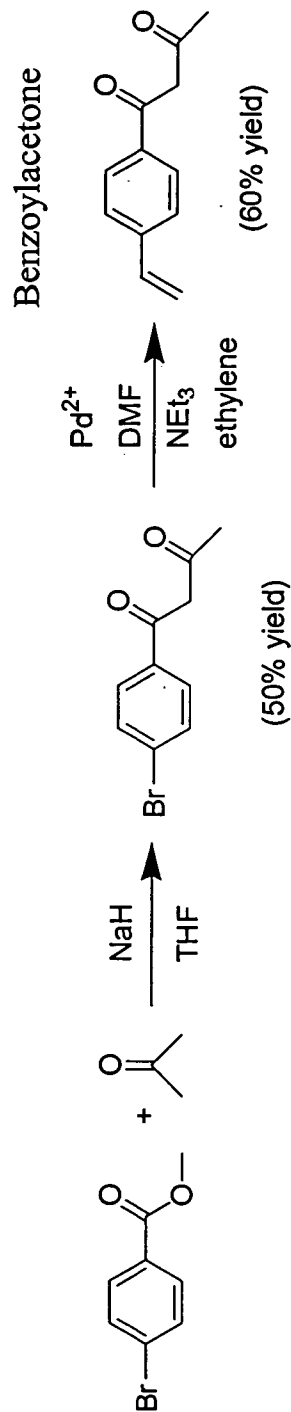
(75% yield)



(43% yield)

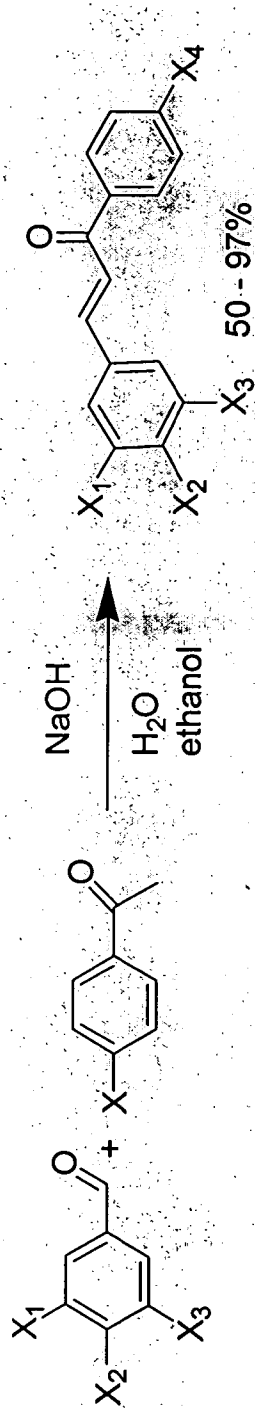
**1**

(41% yield)



(60% yield)

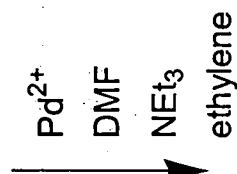
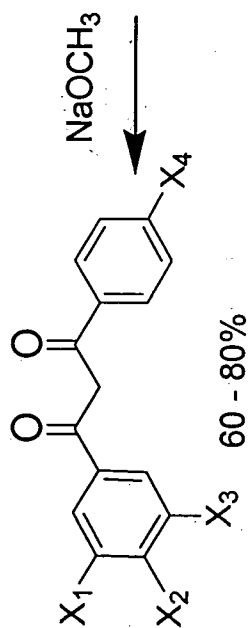
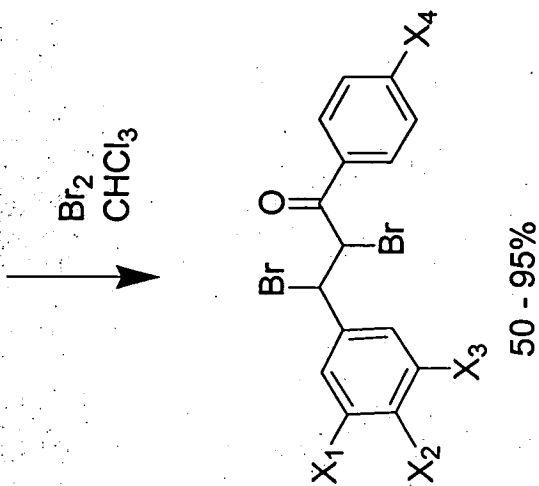
**1**



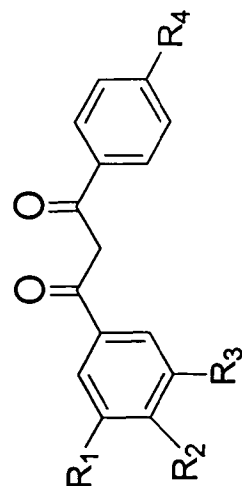
2 H = X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>; Br = X<sub>4</sub>

3 H = X<sub>1</sub>, X<sub>3</sub>; Br = X<sub>2</sub>, X<sub>4</sub>

4 H = X<sub>2</sub>, X<sub>4</sub>; Br = X<sub>1</sub>, X<sub>3</sub>

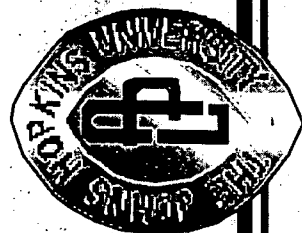


2a H = R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>; vinyl = R<sub>4</sub>  
 3a H = R<sub>1</sub>, R<sub>3</sub>; vinyl = R<sub>2</sub>, R<sub>4</sub>  
 4a H = R<sub>2</sub>, R<sub>4</sub>; vinyl = R<sub>1</sub>, R<sub>3</sub>

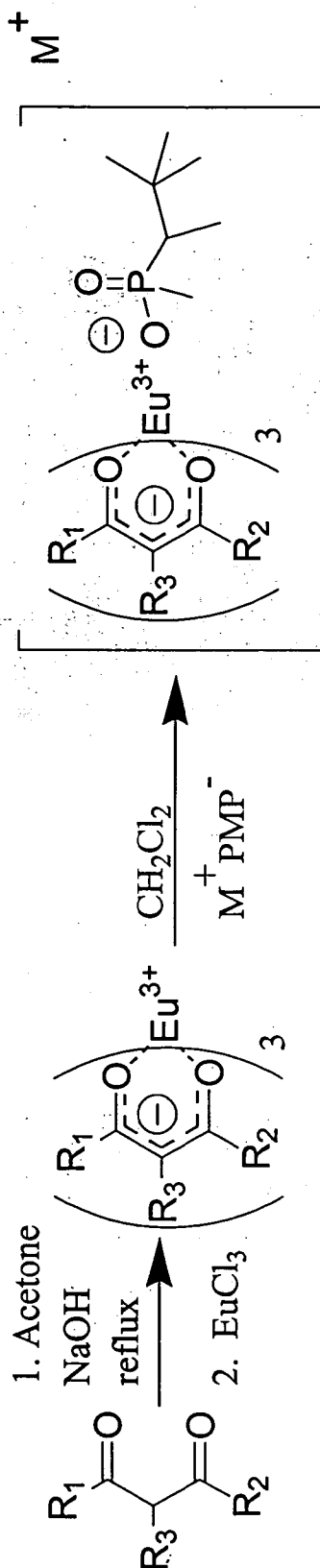


56 - 88%

Dibenzoylmethane (DBM)



# Complex Synthesis

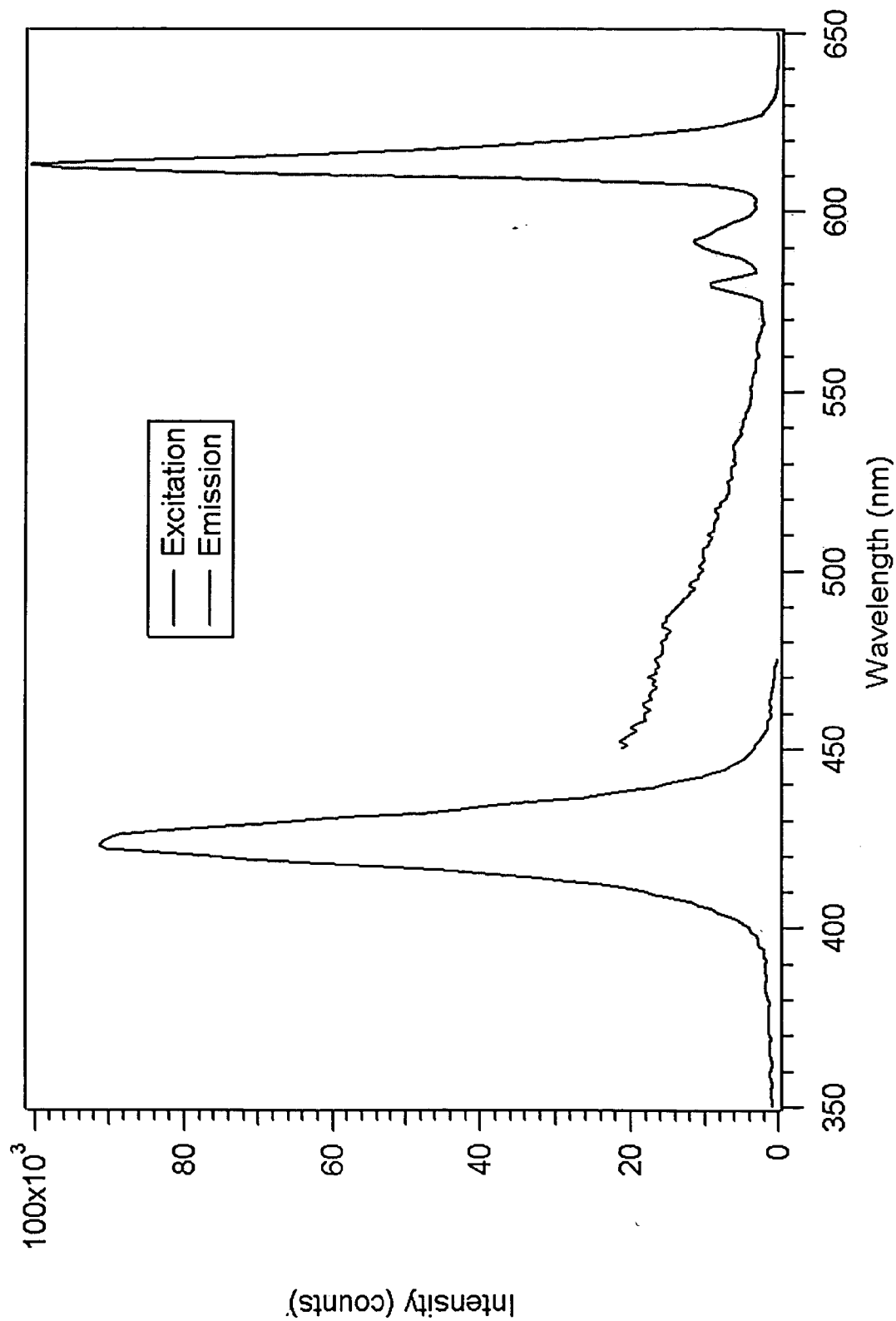


R<sub>1</sub> = 4-vinylphenyl, 3,5-divinylphenyl

R<sub>2</sub> = methyl, phenyl

R<sub>3</sub> = H, allyl, 4-vinylbenzyl

# Tris-(divinyldibenzoylemethanato) Europium (III)







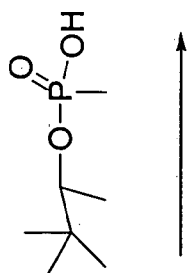
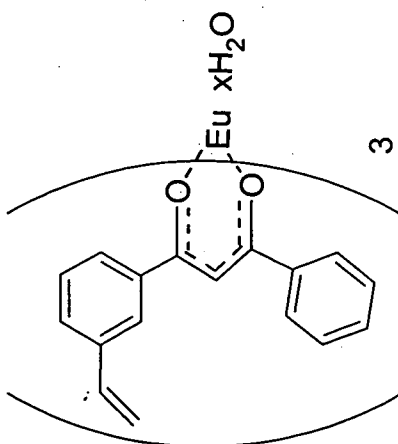
# Self-Assembled Complex

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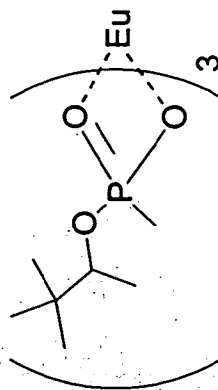
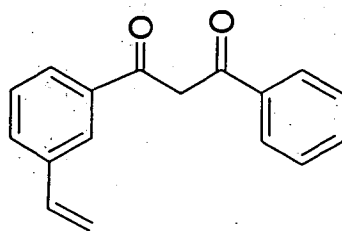
- We are making our complexes using vinyl naphtho trifluoroacetone and vinyl thienyl trifluoroacetone since these complexes can be excited with a 370 nm LED.
- Nonfluorinated ligands have given erroneous results.



# Problems

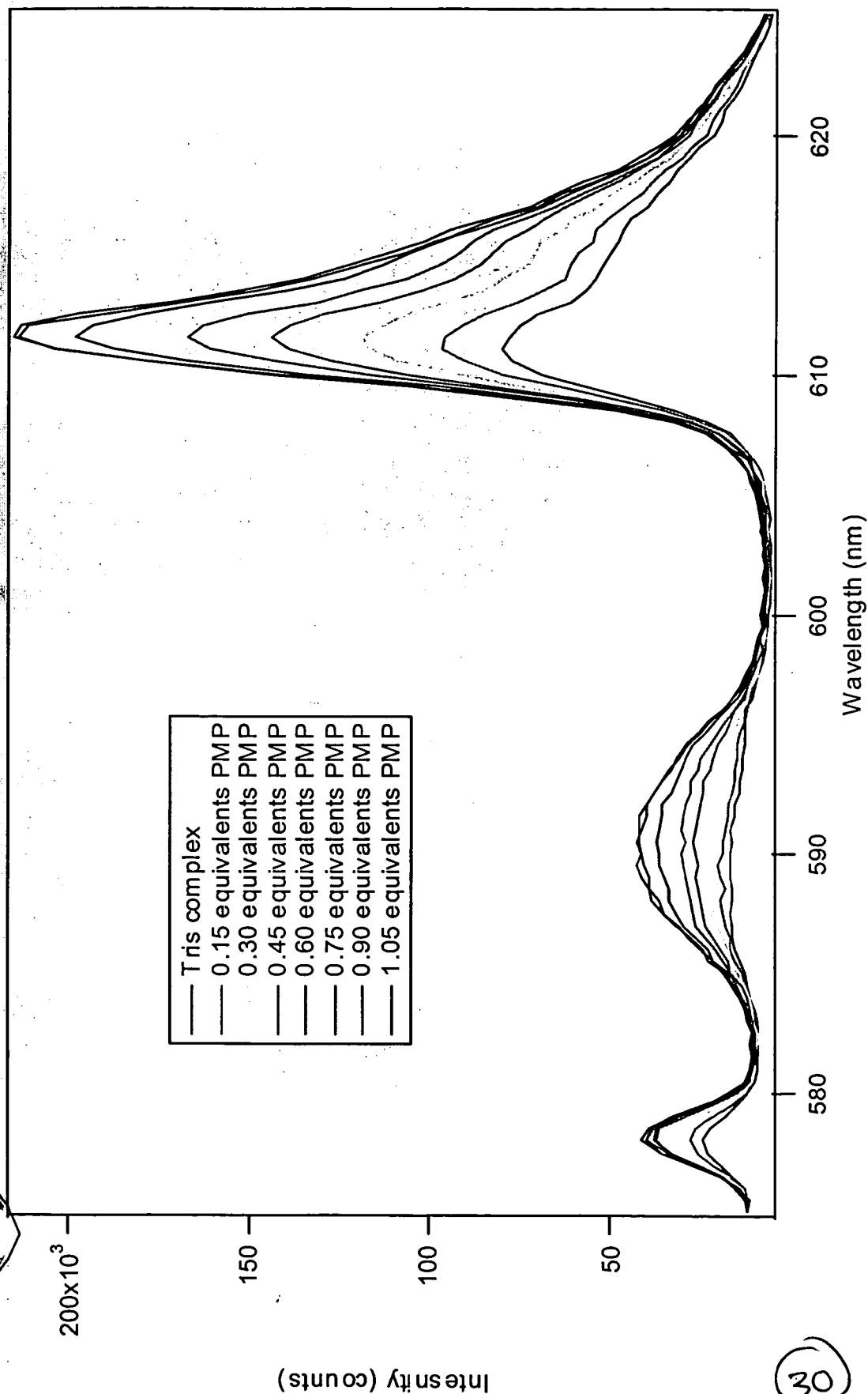


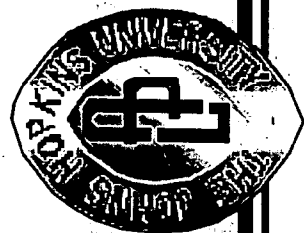
+



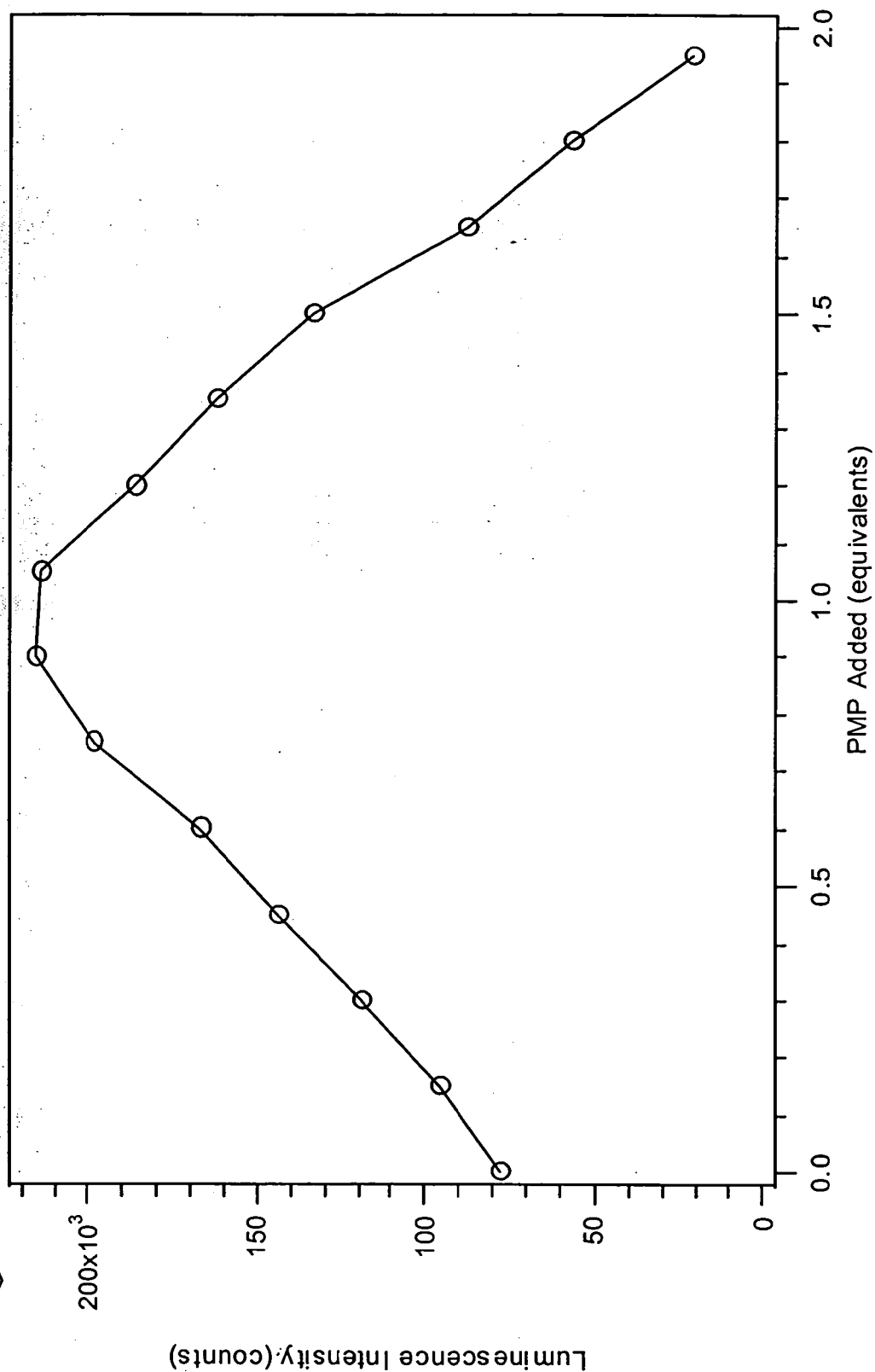


# Titration of $\text{NTFA}_3\text{Eu}$ with PMP





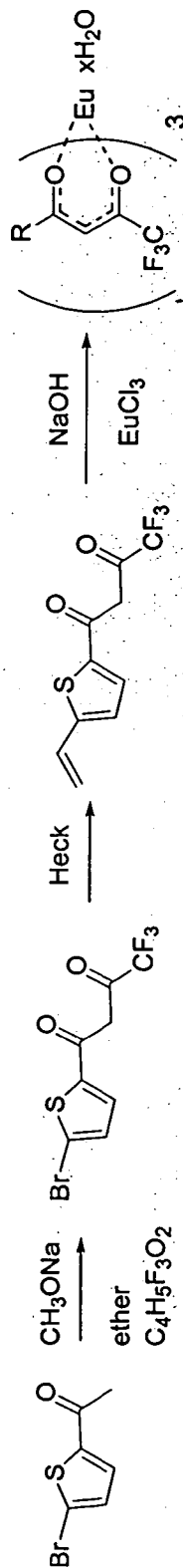
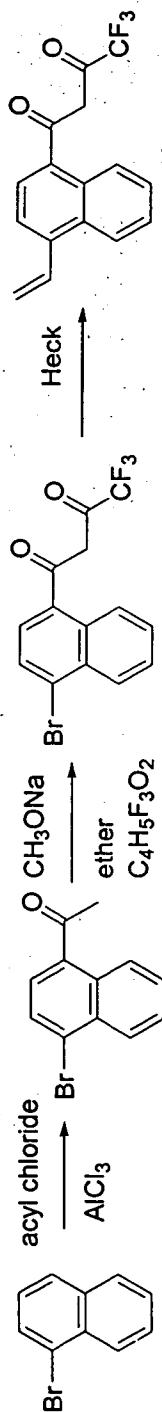
# Luminescence Titration





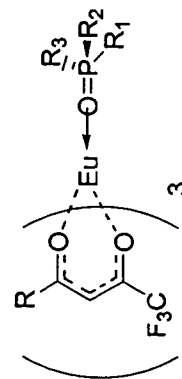
# 2nd Generation Ligands

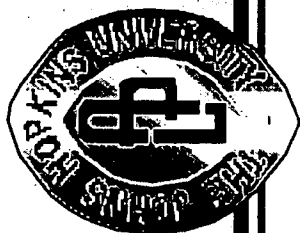
NTFA



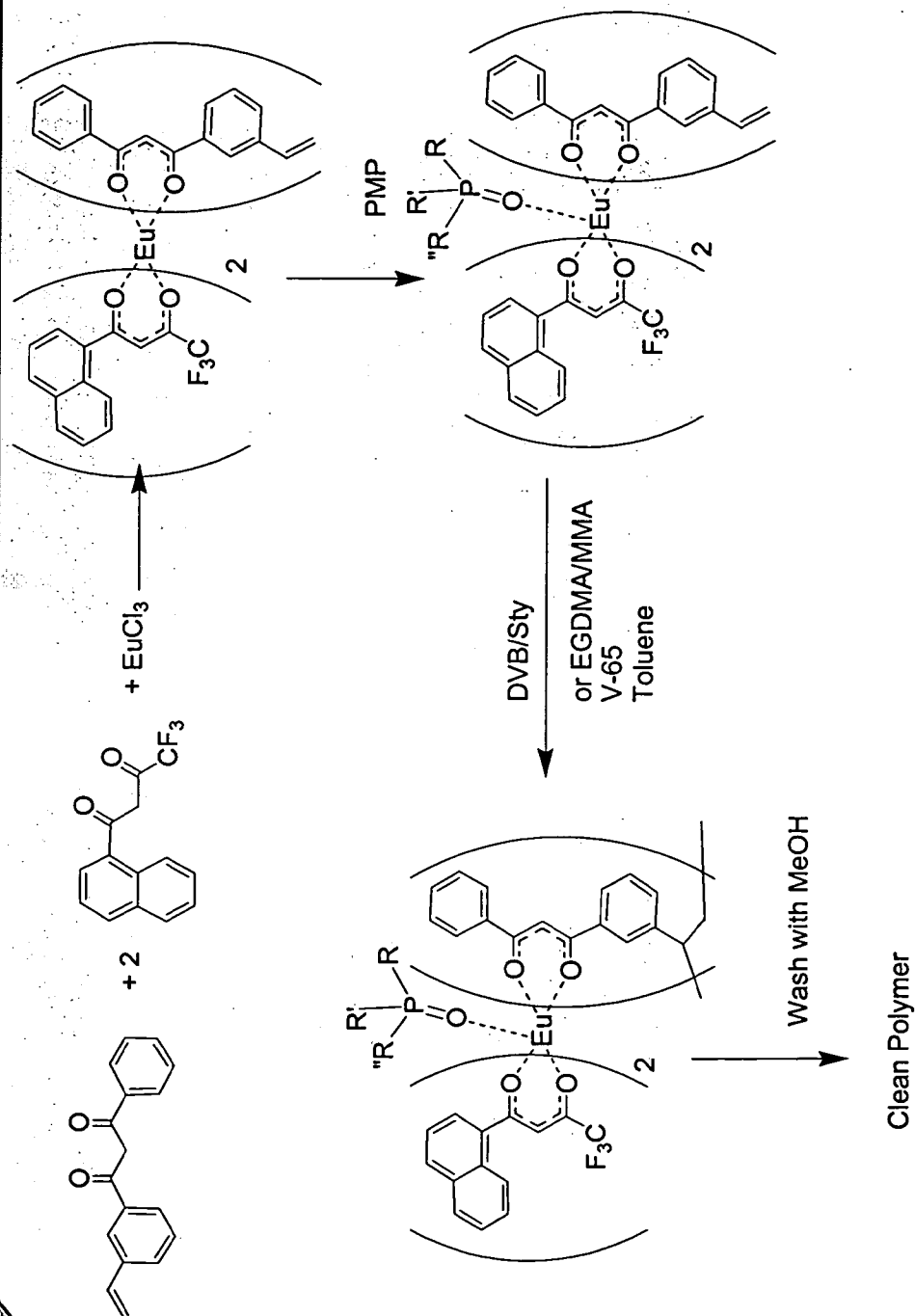
TTFA

R = vinylnaphthyl or vinylthienyl



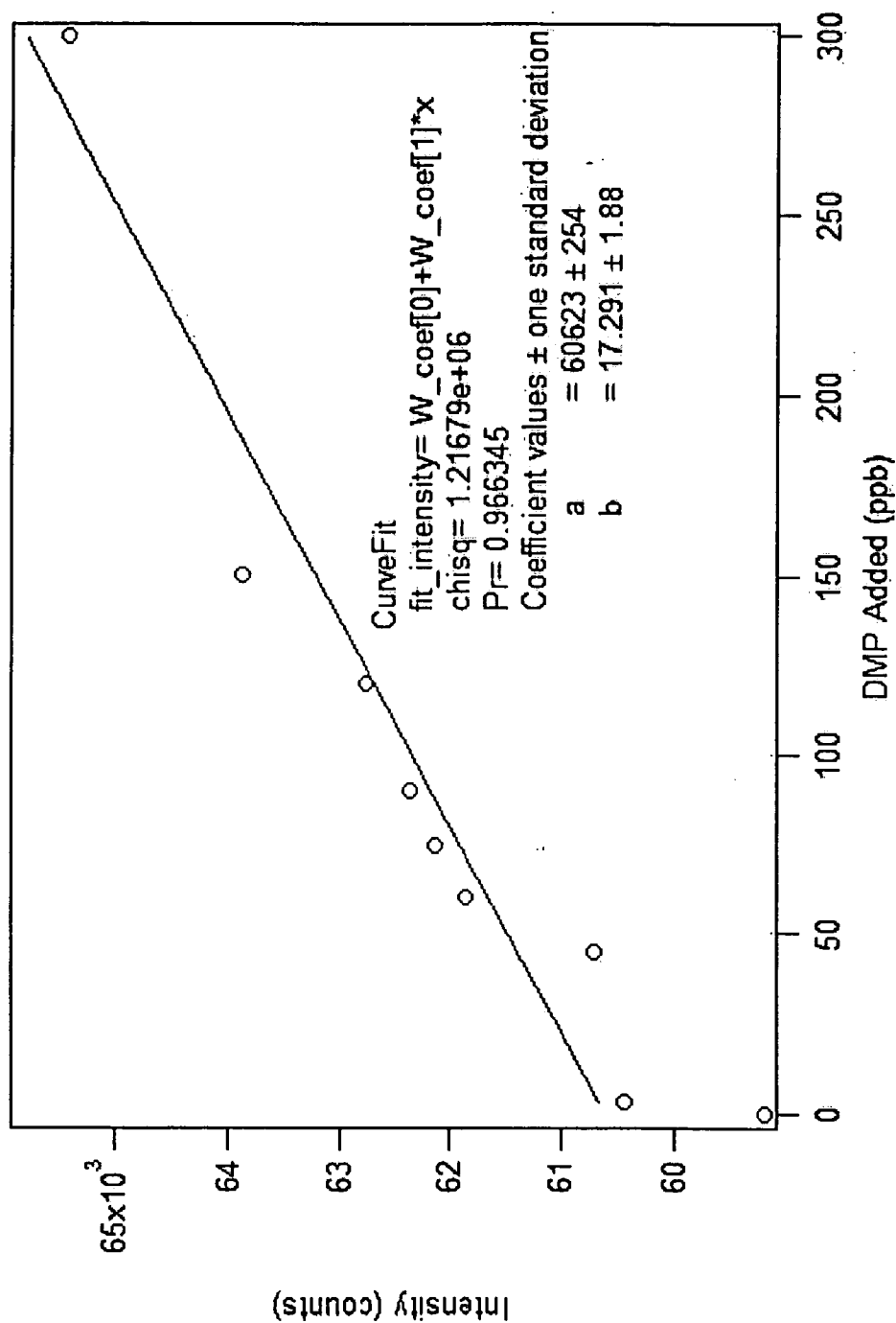


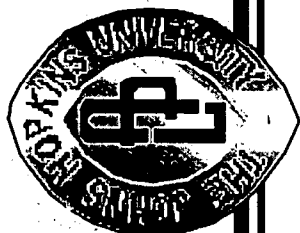
# Mixed Ligand MIPs



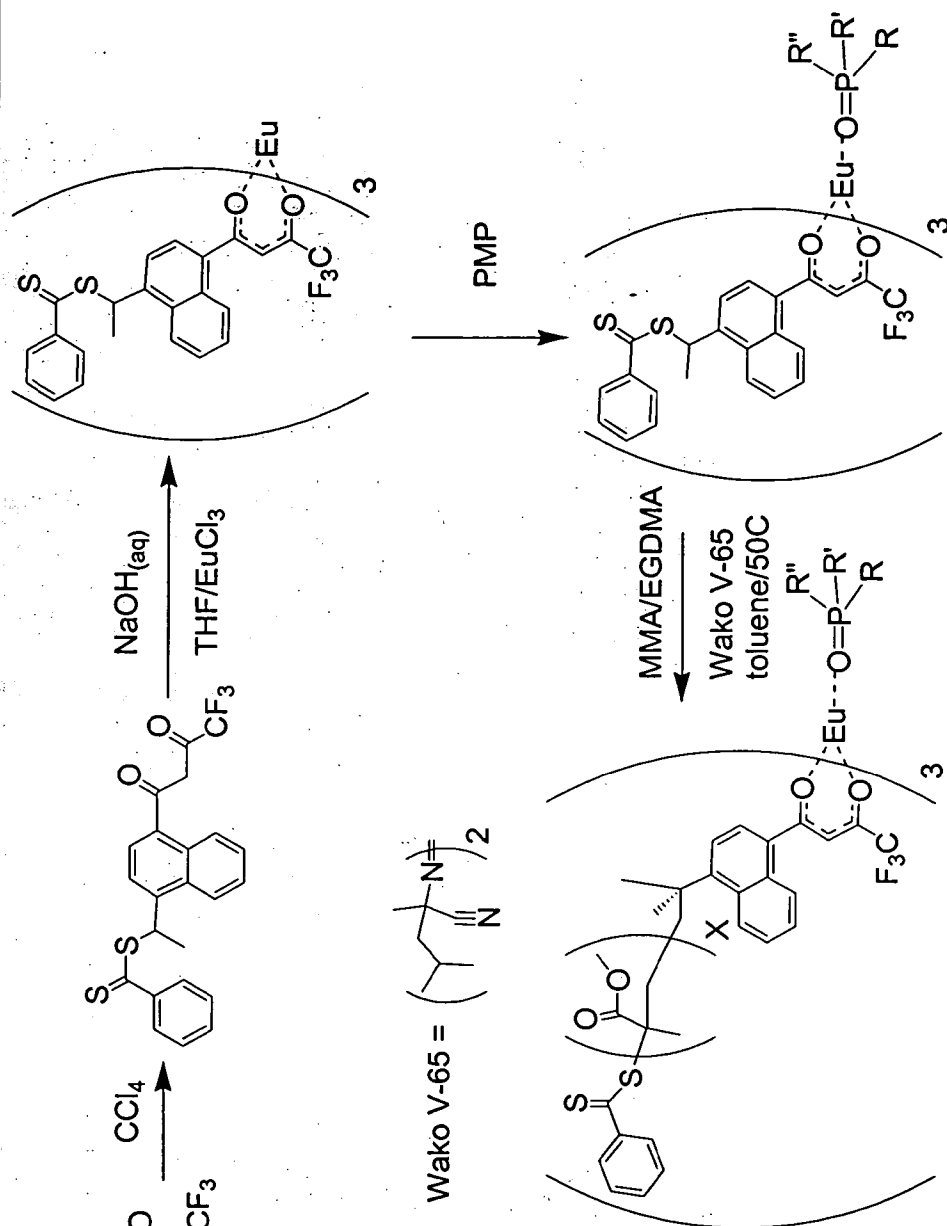


# Mixed Ligand PMP

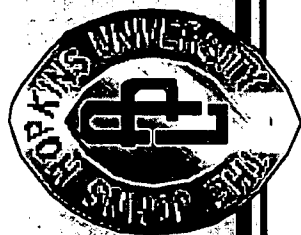




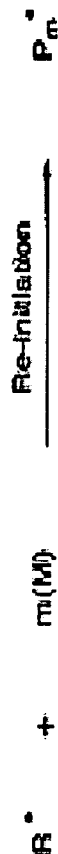
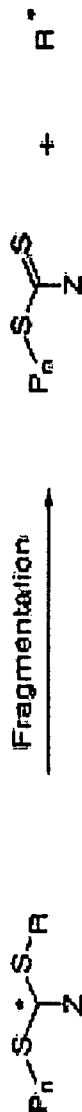
# RAFT



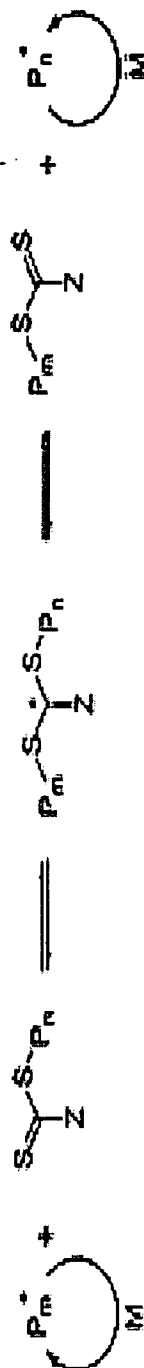




# RAFT Mechanism

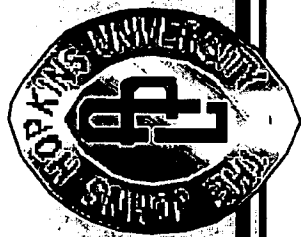


Equilibration

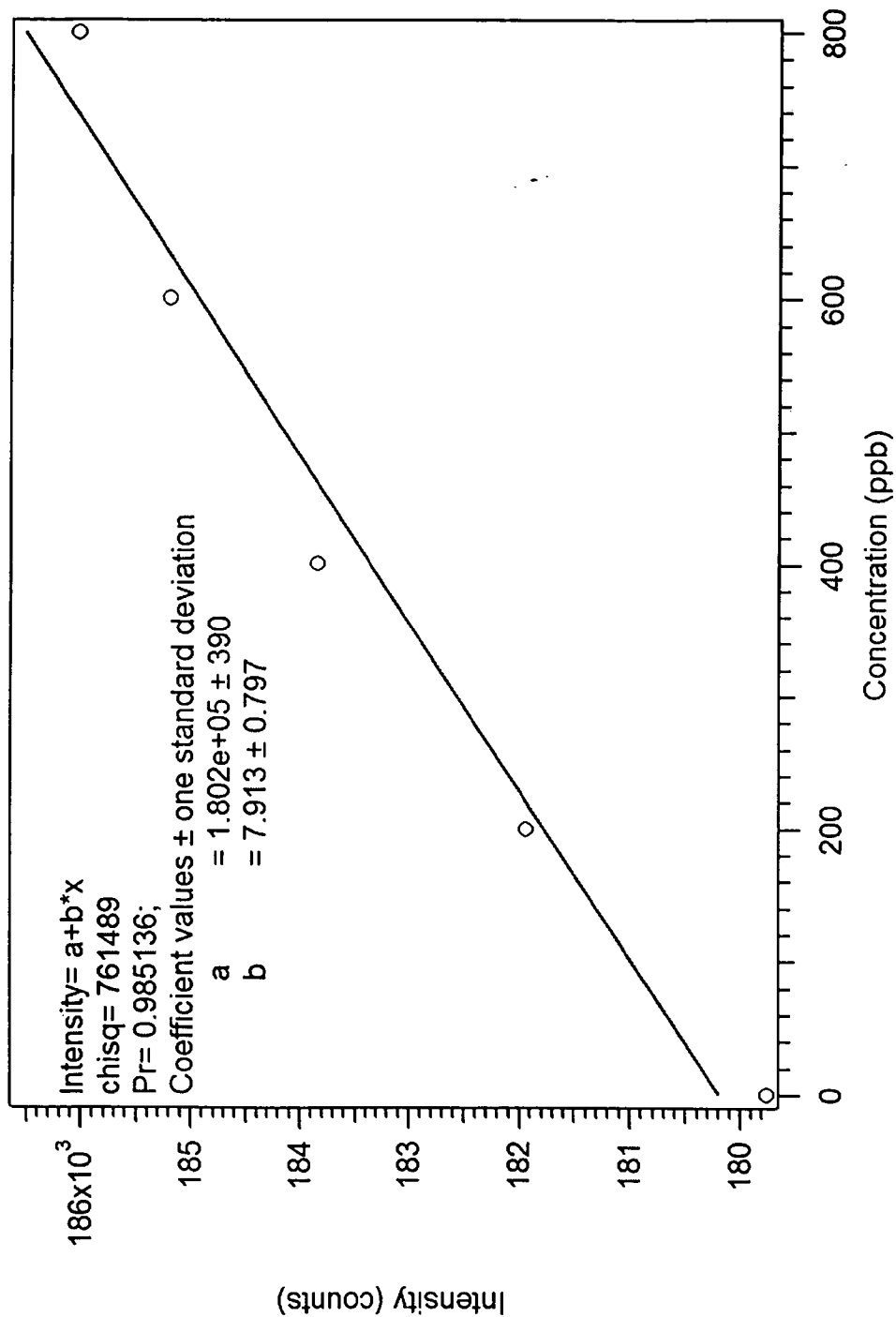


Overall Process





# PMP Imprinted

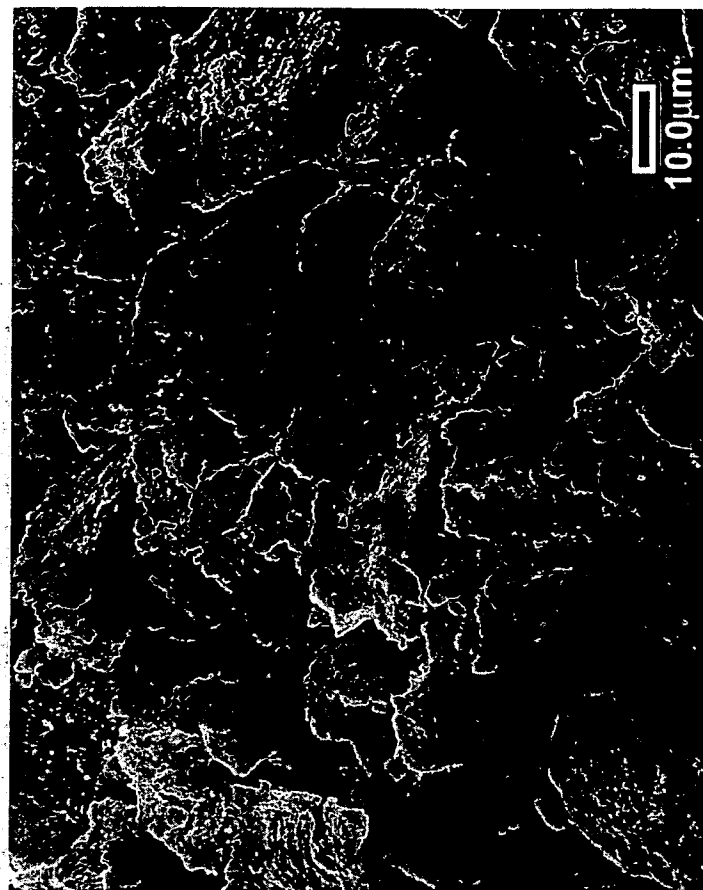
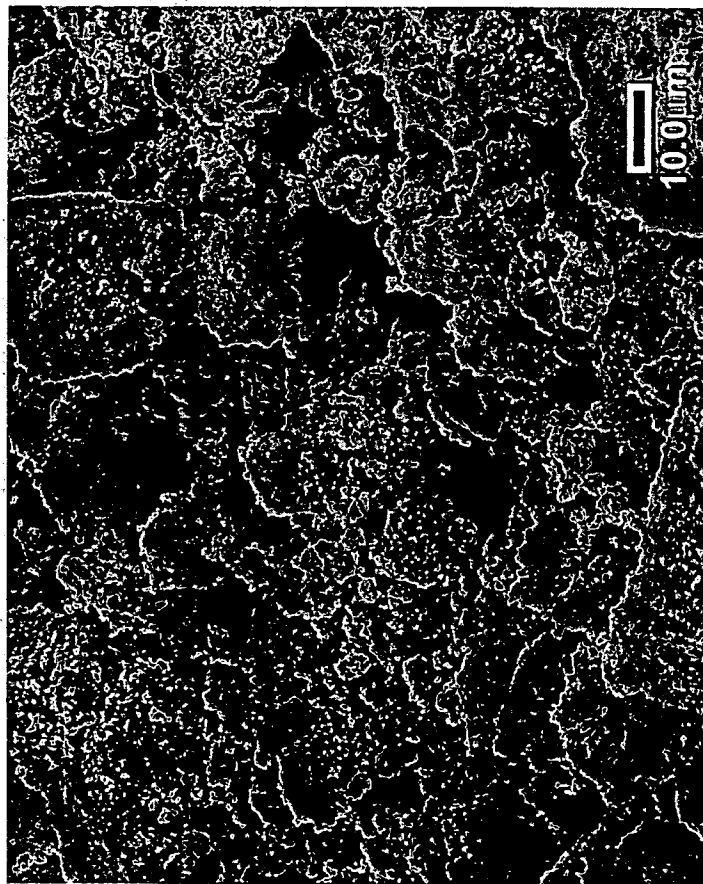




# MIP Morphology

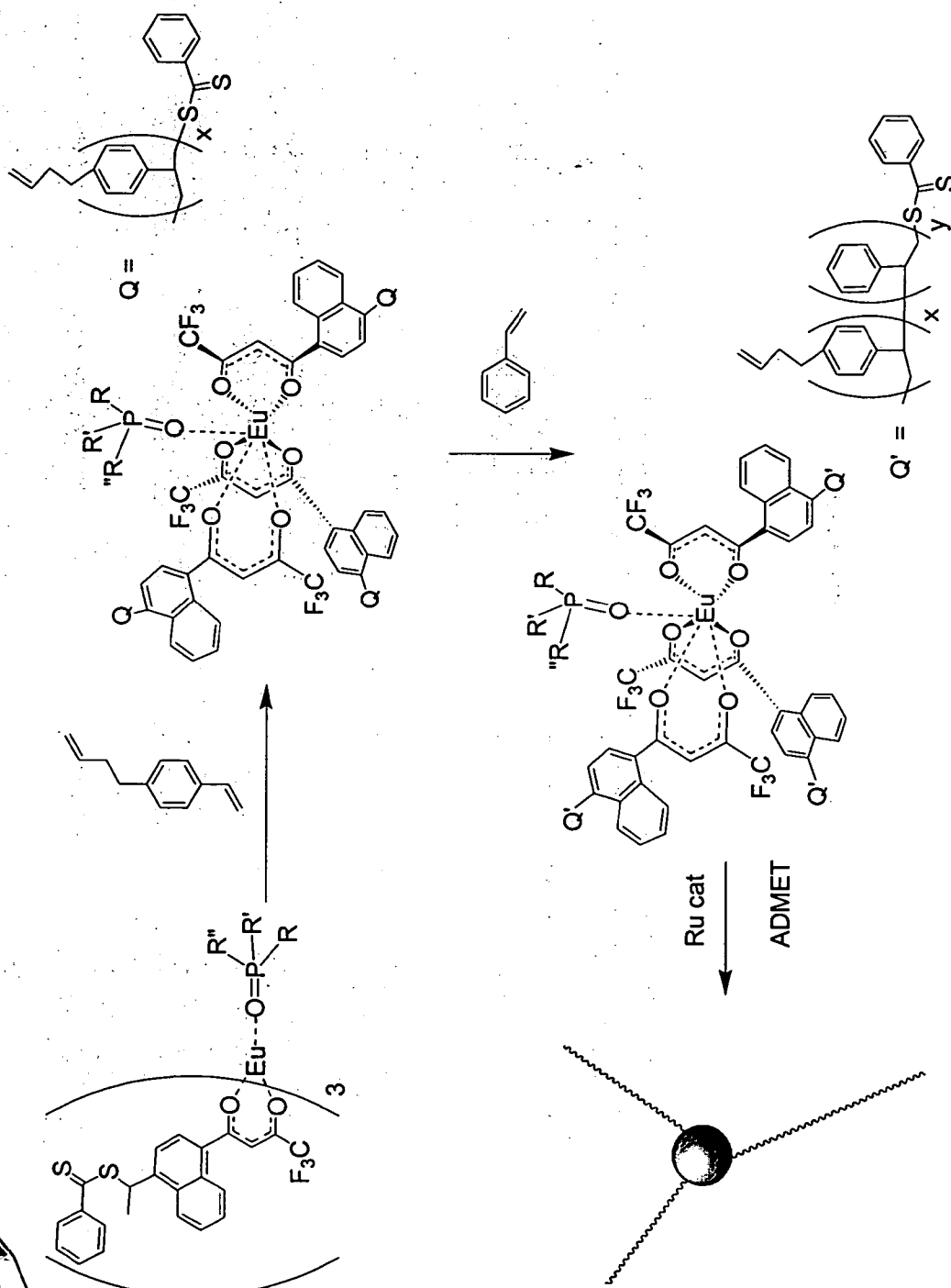
Mixed Ligand

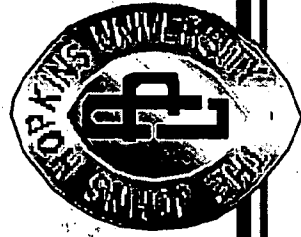
RAFT





# Processible MIP via RAFT





# Conclusions

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- The imprinted polymer detects PMP by a ligand substitution reaction for water.
- The spectrum of the complex is changed by the modulation of the fluorescence of the MIP.
- Ligand displacement has been resolved by use of fluorinated ligands.
- Processability can be imparted by improved polymer architecture



# Acknowledgements

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- SBCCOM
- U. S. Army
- Texas Tech – The Institute for Environmental and Human Health
- Steve Wajer – SEM
- Dr. Manny Uy – Manager